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I. Surgical Session

Volume displacement oncoplastic breast conservation in small breast volume after neoadjuvant chemoimmunotherapy

Valentin Ivanov

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Introduction: Triple negative breast cancer frequently requires extensive surgical excision due to aggressive tumor biology and associated radiological findings such as microcalcifications. In patients with small breast volume, breast conserving surgery following neoadjuvant therapy presents significant reconstructive challenges. Volume displacement oncoplastic techniques allow parenchymal redistribution and reshaping after wide local excision, even in anatomically limited breasts. **Aim:** To present the application of volume displacement oncoplastic surgery in a patient with small breast volume undergoing breast conserving treatment after neoadjuvant chemoimmunotherapy. **Materials and methods:** A 61-year-old woman with small breast volume (cup size B) was diagnosed with a 2.2 centimeter invasive ductal carcinoma, grade 2, triple negative subtype, located between the upper and lower outer quadrants of the breast, accompanied by surrounding microcalcifications extending over 1.3 centimeters. Neoadjuvant chemoimmunotherapy was administere and post-treatment imaging demonstrated complete clinical response; however, residual microcalcifications persisted, necessitating wide surgical excision. **Results and discussion:** Surgical management included extended lumpectomy encompassing both lateral quadrants to ensure oncologic clearance of the microcalcification field, resulting in a significant parenchymal defect. Immediate volume displacement oncoplastic reconstruction was performed through glandular mobilization and tissue rearrangement, despite limited breast volume. Final histopathology confirmed tumor-free resection margins and pathological complete response. Adequate breast contour and symmetry were achieved without need for volume replacement. **Conclusion:** Volume displacement oncoplastic techniques can be successfully applied in small breasts following neoadjuvant therapy for triple negative breast cancer. Careful parenchymal redistribution enables oncologically safe wide excision while preserving acceptable aesthetic outcomes, emphasizing the role of tailored surgical strategies.

Keywords: *triple negative breast cancer, volume displacement oncoplastic surgery, breast conserving surgery, microcalcifications, pathological complete response*

Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype of the disease characterized by lack of expression of estrogen and progesterone receptors and HER2, as well as more frequent cases of high proliferative activity. Its clinical course is characterized by rapid evolution and an increased risk of early recurrence.

In addition to tumor size, associated imaging features are also important for surgical management. The presence of microcalcifications often reflects a widespread intraductal component and necessitates a wider resection, even in the presence of a good response to systemic therapy. This places significant limitations on the organ-conserving approach, especially in patients with small breast volumes, in whom the ratio between resected and residual volume is unfavorable.

The introduction of neoadjuvant chemoimmunotherapy has led to significant advances in the treatment of TSCLC. The addition of pembrolizumab to standard chemotherapy regimens has shown a significant increase in the rate of pathological complete response (pCR), as demonstrated in the Keynote-522 trial [1]. Achieving pCR is associated with improved survival and allows for a more conservative surgical approach [2].

Modern oncoplastic surgery offers opportunities to expand the indications for organ-preserving treatment. Volume-redistributing techniques are based on mobilization and remodeling of the existing glandular parenchyma. However, their application in small breast volumes requires precise assessment of tissue reserve and careful planning of resection lines and the reconstructive stage [3].

Purpose

To present the application of a volume-redistributive oncoplastic approach in a patient with a small breast volume after neoadjuvant chemoimmunotherapy for triple-negative breast cancer.

Materials and methods

A 61-year-old patient with small breast volume (cup) is presented (size B), without significant comorbidities and with good functional status. An invasive ductal carcinoma measuring 2.2 cm, G 2, triple negative subtype was diagnosed.

The tumor is located in the lateral part of the breast, at the border between the upper and lower outer quadrants, and there is also a zone of microcalcifications extending approximately 1.3 cm around the tumor. This finding suggests the possible presence of a concomitant intraductal component and requires oncologically wider excision. (Fig. 1, 2)

No marking of the tumor bed with a clip was performed before starting systemic treatment. Neoadjuvant treatment was performed according to the Keynote-522 protocol, including combined chemoimmunotherapy.

After completion of treatment, imaging studies showed complete clinical response, but microcalcifications persisted. The lack of a pre-placed clip necessitated the use of an alternative localization approach. Intraoperative orientation was achieved by ultrasound visualization of the calcified area and comparison with previous imaging studies.

A wide local excision was planned, including a significant portion of the lateral quadrants. Given the small volume of the breast, a volume-redistributive oncoplastic approach was chosen to optimally utilize the available glandular parenchyma.

Results

The resection performed covered a significant volume of the lateral half of the breast, which resulted in a significant parenchymal defect. (Fig. 3, 4)

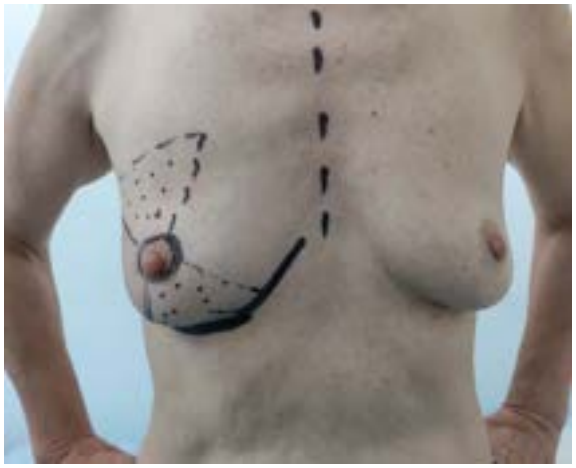

Figure 1.

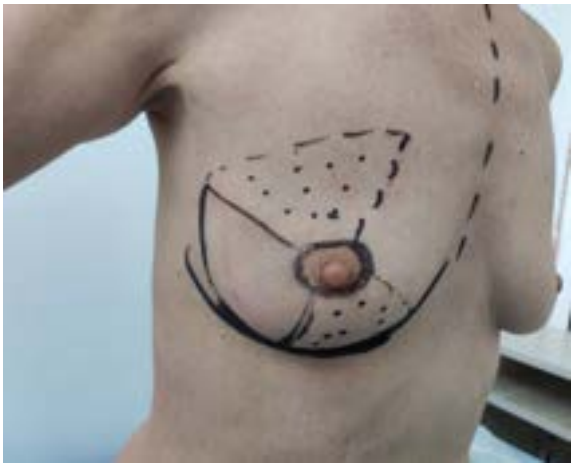

Figure 2.

Figure 3.

Figure 4.

The reconstructive stage included mobilization of the residual glandular parenchyma by separation from the underlying structures and its redistribution to the defect area. Particular attention was paid to preserving vascularization and avoiding tension in the tissues. The skin contralateral to the tumor location was de-epithelialized and the breast was freed from the inframammary fold. The blood supply to the AMC, coming from the 3rd, 4th, and 5th intercostal perforators, was preserved.

Through careful remodeling, restoration of the breast contour was achieved, despite the limited amount of available tissue. (Fig. 5, 6, 7)

Figure 5.

Figure 6.

Figure 7.

Histological examination showed no residual tumor and confirmed a complete pathological response (ypT 0) as well as clean resection lines.

The postoperative period was uneventful. A stable aesthetic result with acceptable symmetry was achieved, without the need for additional interventions.

Discussion

The presented case illustrates the possibilities and limitations of volume-redistributive oncoplastic surgery in patients with small breast volume.

Neoadjuvant chemoimmunotherapy significantly improves the pCR rate, allowing for a reduction in surgical volume and increasing the likelihood of organ-sparing treatment [1, 2]. However, persistent microcalcifications often require wider resection, which may compromise the aesthetic outcome.

The lack of a clip is a significant disadvantage, as it limits the accuracy of localization. In such cases, ultrasound guidance combined with imaging analysis remains a reliable method for intraoperative guidance, although with higher demands on the surgeon's experience.

Volume-redistributing techniques have the advantage of avoiding donor morbidity and preserving local anatomy. However, in small breast volumes they require a careful balance between oncological radicality and aesthetic outcome.

Compared to volume replacement techniques that use additional tissue, volume-redistribution approaches rely entirely on the available parenchyma, making them more suitable for limited defects but more challenging for extensive resections.

Available evidence shows that oncoplastic surgery improves quality of life and patient satisfaction without compromising oncological control [3, 4].

Conclusion

Volume-redistributive oncoplastic surgery can be successfully applied in carefully selected patients with small breast volume after neoadjuvant chemoimmunotherapy.

Even when a wide resection is required and in the absence of prior marking with a clip, ultrasound guidance allows for adequate surgical management. The individualized approach and precise parenchymal remodeling allow for oncological radicality while maintaining a good aesthetic result.

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Volume replacement oncoplastic breast conservation after neoadjuvant chemoimmunotherapy in locally advanced basaloid triple negative breast cancer

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Introduction: Locally advanced basaloid triple negative breast cancer (TNBC) exhibits aggressive biology and high proliferative activity, often requiring mastectomy. Neoadjuvant chemoimmunotherapy can downstage tumors, enabling breast conserving surgery. Volume replacement oncoplastic techniques restore breast volume after extensive resections while maintaining oncologic safety. **Aim:** To present surgical management of locally advanced basaloid TNBC using volume replacement oncoplastic breast conserving surgery after neoadjuvant chemoimmunotherapy. **Materials and methods:** A 53-year-old postmenopausal woman (ECOG 0) presented with a large right breast tumor involving the lower quadrants and a suspicious axillary lymph node. Imaging classified the lesion as BI-RADS 5. Core biopsy confirmed grade 3 invasive ductal carcinoma, estrogen receptor–, progesterone receptor–, HER2–, Ki-67 80%. PET-CT staged the disease as cT3 cN1 cM0. Germline BRCA testing was negative. Neoadjuvant chemoimmunotherapy per Keynote 522 protocol was administered. **Results and discussion:** After partial response to taxane therapy and completion of anthracycline chemotherapy with pembrolizumab, breast MRI showed complete clinical response. Surgical management included wide local excision of the lower quadrants with sentinel lymph node biopsy. Immediate volume replacement using an inferior intercostal artery perforator flap addressed the anticipated parenchymal deficit. Histopathology revealed fibrosis, hyalinization, and necrosis, with tumor-free margins and five negative sentinel lymph nodes, confirming pathological complete response (ypT0 ypN0). Reconstruction preserved breast shape and symmetry. **Conclusion:** Neoadjuvant chemoimmunotherapy enabled conversion from mastectomy to breast conserving surgery in locally advanced basaloid TNBC. Volume replacement oncoplastic reconstruction using an inferior intercostal artery perforator flap effectively restored breast volume after extensive resection, supporting its role in multidisciplinary management.

Keywords: *triple negative breast cancer, neoadjuvant chemoimmunotherapy, volume replacement oncoplastic surgery, pathological complete response, inferior intercostal artery perforator flap*

Introduction

Triple-negative breast cancer is a heterogeneous group of tumors characterized by the lack of expression of estrogen (ER), progesterone (PR) receptors, and HER 2. This subtype accounts for approximately 15–20% of all breast cancers and is associated with an aggressive clinical course, early recurrence, and a poorer prognosis. [1] The basaloid variant is characterized by high proliferative activity and is often diagnosed at a locally advanced stage.

Traditionally, mastectomy has been the preferred treatment for patients with large tumors or unfavorable localization. However, in recent years, neoadjuvant systemic therapy has significantly changed the therapeutic approach. The introduction of immunotherapy, in particular pembrolizumab, in combination with chemotherapy has led to a significant increase in the rate of pathological complete response (pCR), as demonstrated in the Keynote-522 trial [2].

Achieving pCR is associated with a better long-term prognosis and has direct implications for surgical planning, allowing for the transition to organ-sparing surgery in patients initially indicated for mastectomy [3].

In parallel, the development of oncoplastic surgery has expanded the possibilities for organ-preserving treatment. Volume-sparing techniques, including perforating flaps, allow for wide resections while preserving the shape and symmetry of the breast, especially for tumors located in the lower quadrants [3].

Purpose

To present the multidisciplinary approach to the treatment of a patient with locally advanced basaloid triple-negative breast carcinoma using neoadjuvant chemoimmunotherapy followed by volume-replacement oncoplastic organ-sparing surgery using a flap of inferior intercostal artery perforators.

Materials and methods

A 53-year-old postmenopausal patient, without comorbidities, with an overall ECOG status of 0, is presented. Clinically, a tumor formation was detected in the lower quadrants of the right breast, accompanied by a suspicious axillary lymph node.

Imaging studies (mammography and ultrasound) classified the lesion as BI – RADS 5. Core needle biopsy confirmed invasive ductal carcinoma G 3 with basaloid phenotype (ER-, PR-, HER 2-, Ki-67 80%). (Fig. 1, 2)

PET / CT staging revealed disease at stage cT 3 cN 1 cM 0. Genetic testing for BRCA 1/2 mutations was negative.

Neoadjuvant treatment was performed according to the Keynote-522 protocol, including a combination of carboplatin, paclitaxel, and pembrolizumab, followed by an anthracycline regimen with continued immunotherapy.

Evaluation of the therapeutic response by contrast mammography and magnetic resonance imaging showed a complete clinical response (ycT0 ycN0).

In view of the achieved effect, a decision was made for organ-preserving surgery. (Fig. 3)

Figure 1.

Figure 2.

Figure 3.

A wide local excision of the lower quadrants with sentinel biopsy was performed. (Fig. 4, 5)

Figure 4.

Figure 5.

Due to the expected significant volumetric defect, immediate reconstruction was performed using an inferior intercostal artery perforator (ICAP) flap.

Results

The surgical intervention was uneventful. The reconstruction with the ICAP flap provided sufficient tissue volume to repair the defect and preserve the contours of the breast.

Permanent histological examination showed fibrosis, hyalinosis and necrosis without residual tumor, confirming a complete pathological response (ypT 0). The five sentinel lymph nodes examined did not contain metastases (ypN 0).

The postoperative period was smooth, with good wound healing and a satisfactory aesthetic result. (Fig. 6)

Figure 6.

Discussion

The presented clinical case illustrates the current trend towards a personalized and multidisciplinary approach in the treatment of triple-negative breast cancer.

Neoadjuvant chemoimmunotherapy significantly improves the rate of pCR, especially in patients with high-risk disease [2]. Achieving pCR is associated with improved survival and allows for a reduction in the volume of surgical intervention [3].

In this context, organ-conserving surgery is established as an oncologically safe alternative to mastectomy in carefully selected patients, even with initially locally advanced disease [5].

Oncoplastic techniques, and in particular volume replacement approaches, expand the indications for organ-preserving treatment. They are particularly valuable in large resections or in tumors located in the lower quadrants, where the risk of deformity is significant.

The ICAP flap is a reliable reconstructive method that provides well-vascularized tissue without muscle damage and with minimal donor morbidity. Its anatomical proximity makes it particularly suitable for defects in the lower half of the breast [6].

Regarding the axillary stage, in patients with a good response to neoadjuvant therapy, sentinel biopsy may be an adequate alternative to axillary dissection, subject to the relevant selection criteria [7].

All this emphasizes the importance of an integrated approach, in which the optimal combination of systemic treatment, surgery, and reconstruction leads not only to oncological control, but also to a better quality of life.

Conclusion

Neoadjuvant chemoimmunotherapy can lead to significant reduction in tumor volume and allow conversion from mastectomy to organ-conserving surgery in patients with locally advanced basaloid triple-negative breast cancer.

Volume-replacement oncoplastic reconstruction with an ICAP flap provides effective restoration of breast volume after extensive resection and represents a valuable tool in modern multidisciplinary treatment.

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Risk-reducing nipple-sparing mastectomy in BRCA1 mutation carriers: surgical challenges in a young patient with large breast volume

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Introduction: Carriers of pathogenic BRCA1 mutations have a substantially increased lifetime risk of developing breast cancer. Risk-reducing mastectomy significantly lowers cancer incidence and mortality. Nipple-sparing mastectomy with immediate reconstruction has become an accepted option; however, patients with large breast volume and minimal subcutaneous tissue present specific reconstructive challenges. **Aim:** To review surgical considerations in BRCA1 mutation carriers and present a case of prophylactic nipple-sparing mastectomy in a young patient with unfavorable anatomical characteristics. **Materials and methods:** A 27-year-old woman was referred for genetic testing after her mother developed bilateral breast carcinoma. Germline testing confirmed a BRCA1 mutation. The patient had no evidence of malignancy and requested risk-reducing surgery. Clinical assessment revealed large breasts (cup size D) with extremely thin subcutaneous tissue (approximately 4 millimeters). Bilateral prophylactic subcutaneous mastectomy with preservation of the nipple–areola complexes was performed, followed by immediate implant-based reconstruction using 350 cubic centimeter Mentor implants reinforced with TiLOOP Bra Pocket meshes. **Results and discussion:** Large breast volume combined with minimal subcutaneous fat increases the risk of flap ischemia, implant visibility, rippling, and nipple–areola complex compromise. Despite these limitations, careful surgical technique and mesh-assisted implant stabilization allowed satisfactory aesthetic outcome and preservation of breast contour. The case highlights the importance of individualized planning and meticulous soft tissue handling in high-risk patients. **Conclusion:** Risk-reducing nipple-sparing mastectomy is feasible in young BRCA1 carriers, even in anatomically challenging cases, when supported by appropriate reconstructive strategies. Multidisciplinary assessment remains essential to optimize oncologic safety and aesthetic results.

Keywords: *BRCA1 mutation, prophylactic mastectomy, nipple-sparing mastectomy, implant reconstruction, TiLOOP mesh*

Introduction

Approximately 3–5% of breast cancer patients have identified genetic mutations [1]. Carriers of pathogenic mutations in the BRCA1 gene are at significantly increased risk of developing breast cancer, with a cumulative lifetime risk of up to 65% [2]. In addition to the high incidence, the disease in these patients often manifests at a younger age and is characterized by a more aggressive clinical course. In this regard, prophylactic (risk-reducing) mastectomy is established as the most effective strategy for significantly reducing the incidence of the disease and its associated mortality [3, 4].

BI – RADS type D breast density is characterized by a predominance of fibroglandular tissue and minimal fat content. This type occurs in approximately 10% of women, may make tumors difficult to detect on mammography, and is associated with an approximately two-fold increased risk of developing carcinoma compared with type B [5, 6].

With the development of reconstructive surgery and the increased expectations of patients, the modern approach is aimed not only at oncological certainty, but also at optimal aesthetic outcome and quality of life. Nipple-sparing mastectomy (NSM) with immediate reconstruction has become the preferred method in carefully selected patients, since the preservation of the overlying skin and the nipple-areola complex is essential for body image and psycho-emotional comfort [7].

However, NSM remains a technically challenging procedure, especially in patients with unfavorable anatomical features. The

large volume of glandular parenchyma at the expense of adipose tissue in the breast is associated with longer and thinner skin flaps, which increases the risk of compromised perfusion. On the other hand, thin subcutaneous fat reduces implant protection and increases the risk of visibility, palpability, and rippling [8, 9].

These factors require individualized surgical planning and precise execution to achieve a balance between oncological radicality and reconstructive outcome.

Purpose

To analyze the surgical features, potential risks, and reconstructive options of prophylactic nipple-sparing mastectomy in a young patient with a BRCA 1 mutation and unfavorable anatomical features.

Materials and methods

We present a 27-year-old female patient referred for genetic testing due to a family history of bilateral breast cancer in her mother. Genetic testing confirmed the presence of a pathogenic mutation in the BRCA 1 gene.

Imaging studies (mammography and ultrasound) and clinical examination did not reveal any evidence of a malignant process. After discussion in a multidisciplinary team and after informed consent from the patient, a decision was made for prophylactic bilateral mastectomy.

Clinical assessment reveals breasts with large volume (cup size C) and extremely thin subcutaneous fat (0.25–0.4 mm). (Fig. 1)

This anatomical profile creates a risk of necrosis of the areomammillary complex or overlying skin in the event of overly cautious surgical dissection above the glandular parenchyma. On the other hand, leaving healthy glandular tissue to avoid ischemic complications increases the risk of developing oncological disease.

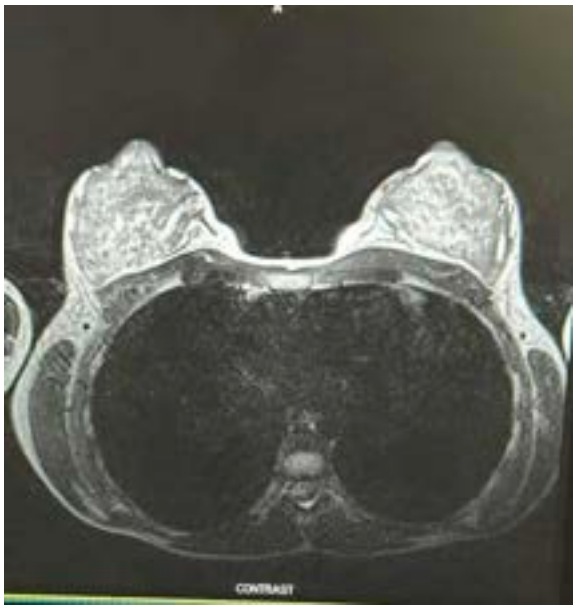

Figure 1.

A bilateral subcutaneous mastectomy was performed with preservation of the areola-mammary complex. Reconstruction was performed in one step with anatomical silicone implants (350 cc, Mentor). To optimize the soft tissue coverage and implant stability, a synthetic titanium-coated polypropylene mesh (TiLOOP) was used. Bra Pocket).

The operative technique involves sparing dissection in the anatomical fascial planes, with maximum preservation of the subcutaneous vascular plexuses and minimization of thermal damage.

Results

The surgical intervention proceeded without intraoperative complications. The viability of the skin flaps and the areola-mammary complex was preserved.

No signs of ischemia, necrosis, or infection were observed in the early postoperative period. The use of the synthetic mesh contributed to implant stabilization, improved soft tissue coverage, and reduced tension on the skin flaps [10].

The aesthetic result was good, with a well-formed contour and acceptable symmetry. Minimal implant visibility was

observed, with no clinically significant rippling.

Histological examination did not reveal the presence of a malignant process.

Discussion

BRCA1 mutation carriers has been shown to be an effective method for reducing the risk of developing breast cancer [3, 4]. NSM has been established as a safe approach with good oncological and aesthetic outcomes when applied to carefully selected patients [7, 11].

The main challenge in this type of surgery remains the perfusion of the skin flaps. The thickness of the flap is a key factor – too thin flaps increase the risk of necrosis, while too thick ones can lead to residual glandular parenchyma [8].

In patients with predominantly large glandular breast parenchyma and thin subcutaneous tissue, the risk of complications is significantly higher. In these cases, the use of synthetic or biological matrices may improve the reconstructive outcome by providing additional implant support and optimizing soft tissue distribution [10, 12].

Surgical technique is also an important aspect – the correct type of implants, precise dissection, preservation of blood supply, and minimizing thermal trauma are critical to the success of the procedure.

The presented case demonstrates that even under unfavorable anatomical conditions, it is possible to achieve a good result through careful planning and an individualized approach.

Conclusion

Prophylactic risk-reducing mastectomy with preservation of the areola-mammary complex and immediate reconstruction is an effective and feasible approach in young carriers of BRCA 1 mutations.

“D” tick density and minimal subcutaneous tissue, good results can be achieved through careful planning, precise surgical technique, and the use of modern reconstructive tools.

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Methods for improving safety during laparoscopic cholecystectomy

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Introduction: Laparoscopic cholecystectomy is the gold standard for the treatment of symptomatic gallstone disease. While it offers numerous advantages over open surgery, it is associated with a specific risk of serious complications, most notably iatrogenic bile duct injuries. **Aim:** The aim of this study is to analyze and highlight the most effective surgical techniques and standardized protocols required to minimize intraoperative risks and ensure patient safety during cholecystectomy. **Materials and methods:** A comprehensive review of surgical practices and clinical guidelines regarding safe cholecystectomy was conducted. The study focuses on anatomical identification via the "Critical View of Safety", intraoperative cholangiography, and indocyanine green fluorescence imaging in difficult cases. **Results and discussion:** The analysis indicates that the primary cause of bile duct injury is the visual misperception of local anatomy rather than a lack of surgical skill. The routine implementation of the Critical View of Safety significantly reduces the incidence of biliary injuries. IOC and ICG fluorescence imaging serve as essential navigational aids for clarifying anatomy in complex cases. The results demonstrate that blunt dissection, adequate traction of the gallbladder, and careful identification of the cystic duct and cystic artery are paramount. **Conclusion:** Strict adherence to standardized safety steps and precise dissection are essential for preventing iatrogenic injuries. The universal application of the Critical View of Safety is the most reliable method for improving outcomes.

Keywords: *Cholecystectomy, patient safety, bile duct injury*

Introduction

Over the last decade, laparoscopic cholecystectomy has established itself as the "gold standard" for surgical treatment of symptomatic gallstone disease. Thanks to its minimal invasiveness and rapid patient recovery, it is the most commonly performed laparoscopic procedure in general surgery. Despite the undeniable advantages of the method over open surgery, its routine nature, standardized training protocols, and advances in equipment, intraoperative complications remain a serious challenge. For prevention to be effective, it is necessary to classify the complications, and here we will apply the following:

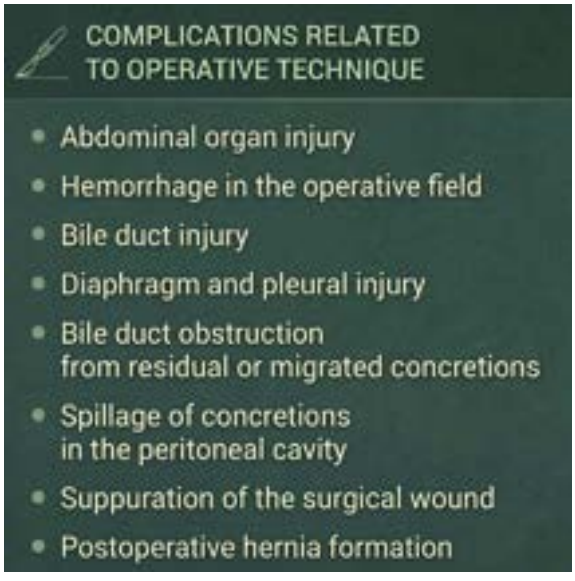

Figure 1. Complications related to operative technique

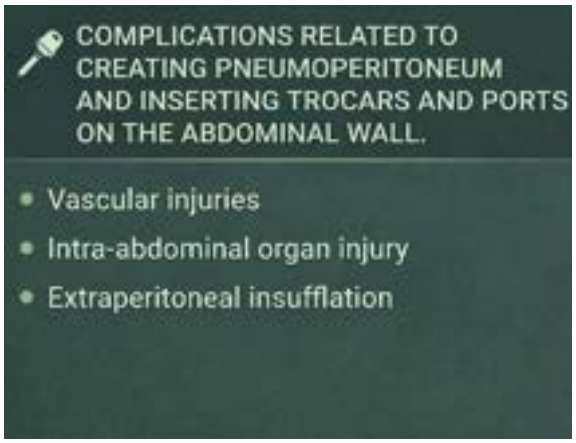

Figure 2. Complications related to creating pneumoperitoneum and inserting trocars and ports on the abdominal wall

The frequency of complications has remained within relatively constant limits in recent years. Contemporary clinical analyses show that the root cause of these incidents most often lies not in the lack of technical surgical skills, but in visual misperception of local anatomy. Prevention of iatrogenic injuries and their serious consequences requires a transition from subjective judgment to strict, routine application of validated methods for anatomical identification. In this context, systematic training, self-improvement, and knowledge of navigation methods are the only path to eliminating iatrogenic risk.

Aim

The aim of the present study is to systematize, analyze, and bring to the forefront the most effective contemporary surgical techniques and standardized protocols for management, whose strict application is necessary to minimize intraoperative risks and guarantee maximum patient safety during laparoscopic cholecystectomy.

Methods

To achieve the stated aim, a review analysis of contemporary clinical guidelines, surgical protocols, and specialized literature on safety in laparoscopic cholecystectomy was conducted. The methodology is built on the following main directions:

- Detailed anatomical review: Based on classical atlases and guidelines. Perfect knowledge of the topography of the extrahepatic biliary tree, Calot's triangle, and their variations is fundamental for the prevention of iatrogenic lesions.
- Analysis of intraoperative risks: Structured on the basis of established monographs on complications in minimally invasive surgery and augmented with current data from evidence-based medicine.
- Systematization of safety strategies: The key technologies for anatomical identification and prevention were investigated, including the Critical View of Safety (CVS) concept, Intraoperative Cholangiography (IOC), and Fluorescence Imaging with Indocyanine Green (ICG).

The present study is entirely review and analytical in nature, is based on retrospective analysis of scientific facts, and does not include direct clinical experiments with patients.

Results

1. Methods for preventing complications related to the creation of pneumoperitoneum and placement of trocars and ports on the abdominal wall

Strict technique with the Veress needle guarantees safe access and prevention of iatrogenic lesions. The patient is placed in a mild Trendelenburg position, which aids gravitational displacement of the abdominal organs in a cranial direction and frees the pelvic area. The puncture is performed in the area immediately above or below the umbilicus, where the abdominal wall is thinnest and the parietal peritoneum is closely adherent to the fascia of the linea alba. To ensure

maximum distance between the abdominal wall and the visceral organs, manual traction is applied by the assistant and operator. The needle is inserted at an angle of 45 degrees to the horizontal line in the direction of the pelvis. Control during passage through the abdominal wall is achieved through tactile and auditory recognition of the two “clicks” (fascia and peritoneum). Before insufflation, the intraperitoneal position must be confirmed through the aspiration test, the “falling drop” test, and manometry.

1.1. Injury to major vessels

To avoid vascular injuries to the major vessels (Aorta, V. Cava, Iliac vessels), the surgeon's forearm must be pressed firmly against the body to prevent the sudden “dropping” of the instrument after overcoming the fascia. The skin incision should be slightly wider than the trocar to eliminate skin resistance. Penetration is performed with controlled rotational movements (180°), not with direct strong pressure. In high-risk patients, the open Hasson method can be applied instead of blind puncture. If the instrument enters a major vessel, it must not be removed but left in place as an anatomical marker for locating the lesion during subsequent emergency laparotomy.

1.2. Prevention of injuries to the blood vessels of the abdominal wall (A. Epigastrica)

Injuries to the abdominal wall often remain latent during the intervention due to the tamponade effect of the trocar cannulas. To ensure safety, diaphanoscopy and continuous visual monitoring are applied when inserting each additional port. Before desufflation, it is critical to perform a final inspection of all incision sites. Reducing the intra-abdominal pressure during this inspection is mandatory, as the pneumoperitoneum can temporarily mask active bleeding.

1.3. Prevention of injuries to hollow abdominal organs

The most at-risk organs during access are the small intestine, colon transversum, stomach, and urinary bladder. In patients with a history of previous laparotomies or inflammatory processes, the high risk of peritoneal adhesions necessitates avoiding “blind” insertion of the Veress needle. In these cases, Palmer's point (in the left hypochondrium) is a safe alternative due to the low frequency of adhesions in this zone. Despite this, the “open Hasson method” remains the “gold standard” for safety, as direct visualization eliminates the risk of perforation of organs adherent to the abdominal wall. During the initial inspection, the surgeon must monitor for the appearance of gas on the surface of the intestines – often the only early sign of microperforation by the needle.

1.4. Prevention of injuries to parenchymal organs

These injuries manifest with massive hemorrhage immediately after insertion. Preventive measures include mandatory preoperative examination for hepatosplenomegaly, as enlarged organs fall within the zones of typical puncture. The use of atraumatic (with a protective mechanism) or optical trocars significantly reduces the risk of lesions. Before desufflation, a 360-degree revision of the abdominal cavity is mandatory to rule out discrete injuries that could manifest clinically in the postoperative period.

1.5. Extraperitoneal insufflation

This includes subcutaneous emphysema, preperitoneal insufflation, pneumomediastinum, pneumothorax, and gas embolism (a rare complication but with high mortality). To avoid these, the rules for access with the Veress needle must be followed.

2. Complications related to operative technique

2.1. Bile duct injury

Although biliary lesions remain a serious clinical challenge, the implementation of systematized surgical techniques significantly improves intraoperative safety.

- The Infundibular Technique

The results show that although the infundibular technique is widely used, it carries the risk of the so-called “false funnel picture”. In the presence of a severe destructive process or large stones in Hartmann's pouch, the common bile duct (CBD) can be tented and mistakenly interpreted as the cystic duct. Data indicate that this is one of the leading causes of complete transection of the common bile duct.

- Critical View of Safety (CVS) – “The Gold Standard”

This is a dissection protocol aimed at eliminating visual illusions. To confirm achievement of CVS, the surgical team must simultaneously visualize the following three components:

1. Clearing of Calot's triangle (Hepatocystic triangle): The space defined by the ductus cysticus, common hepatic duct, and the lower edge of the liver must be freed from all fatty, fibrous, and areolar tissue. The goal is full visualization of only two structures entering the gallbladder. It is important that the common bile duct (CBD) and common hepatic duct are not deliberately sought through dissection (due to high risk), but the space around them should be clear.

2. Release of the lower part of the gallbladder: this step is often skipped by novice surgeons. The lower 1/3 of the gallbladder body must be separated from the liver bed (cystic plate). This ensures that the identified structures actually enter the gallbladder and do not pass behind it (such as the right hepatic duct).

3. Identification of “The two structures”: After completing the previous steps, only the following should be clearly visible:

- **Ductus cysticus** (Cystic duct)
- **Arteria cystica** (Cystic artery)
- Confirmation method: Both structures must be inspected through **360 degrees** (anteriorly and posteriorly) before placing clips.

Double-checking and human factor: CVS must also be confirmed by the assistant. Safety depends not only on technique but also on the psychological disposition of the team. Results emphasize the importance of: accepting advice from colleagues; recognizing the effect of fatigue; and the Hunter team approach (ceasing dissection and waiting with clipping until every team member is convinced of correct identification).

The results of CVS implementation demonstrate: secure identification of d. cysticus and a. cystica, effectiveness in fully releasing the lower third of the gallbladder from the liver bed, and that the inability to achieve a “clean” CVS is an absolute indication for conversion to open surgery or transition to an alternative method (Bail-out).

- Role of Intraoperative Cholangiography (IOC)

Performed through a small incision in the d. cysticus, through which a catheter is inserted and a radiopaque contrast medium is injected. With the help of a C-arm (mobile X-ray), the passage of the contrast is visualized. Data confirm that routine use of cholangiography halves the cases of bile duct injury. It allows visualization of the anatomy in its entirety and early recognition of errors, but requires specific skills for interpretation. There are cases where incorrect reading of the cholangiogram itself leads to an iatrogenic lesion.

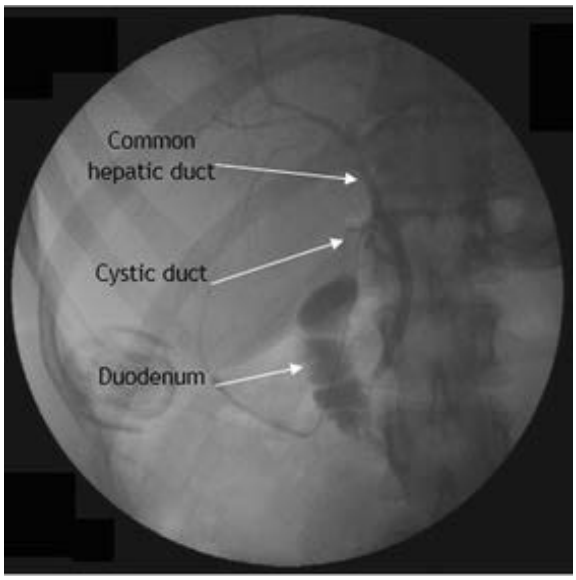

Figure 3. Intraoperative Cholangiogram (IOC)

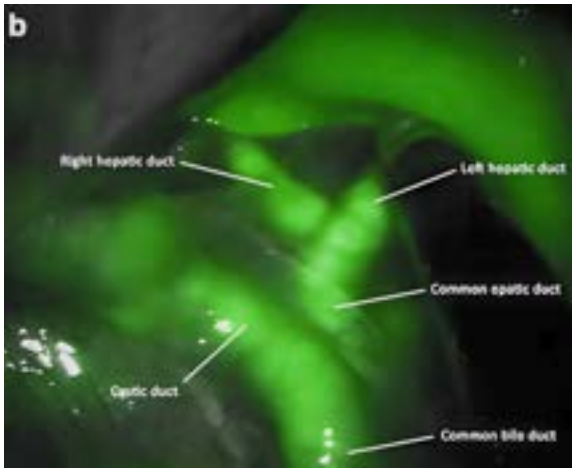

Figure 4. Indocyanine green (ICG)

- ICG Fluorescence Cholangiography – The Most Modern Method, Gaining Enormous Popularity

The patient receives intravenous injection of Indocyanine Green (ICG) a few hours before the operation. The substance is excreted exclusively through the bile. When illuminated with near-infrared light (NIR), the bile ducts start to "glow" in bright green. It is non-invasive because the duct does not need to be cut to insert a catheter. It is performed in real time and the surgeon sees the anatomy during the dissection itself, without stopping the operation for X-ray. It allows visualization of structures even through fatty tissue or severe inflammation, before the dangerous dissection has even begun. Although expensive, methods using indocyanine green (ICG) or methylene blue (introduced via Veress needle) show potential for better visualization, but sufficient statistically significant data on their mass efficacy are still lacking.

- "Bail-out" Strategies – When is it Better to Stop?

A critical moment for safety is the timely recognition of a situation in which the operation becomes too risky. In such cases, the following strategies are applied:

- **Subtotal cholecystectomy:** Applied in a "frozen" Calot's triangle where dissection is impossible. The surgeon opens the gallbladder, evacuates the stones, and removes the accessible part of its wall. A remnant of the infundibulum prevents the risk of injury to the choledochus. The base can be sutured or left open with placement of a drain.
- **Conversion:** Transitioning to open surgery is not a failure but a decision in the name of safety. The inability to achieve a clear CVS is an absolute indication for conversion.
- **Cholecystostomy:** Draining of the gallbladder (often under ultrasound guidance) to control acute inflammation and postpone the operation to a later stage.
- **Antegrade ("Fundus-first") technique:** Dissection begins from the fundus of the gallbladder toward the cystic duct. Used in cases of massive infiltrates in Calot's triangle, where "blind" dissection is dangerous. The method allows working in the safe plane between the gallbladder and the liver, but carries the risk of "traction illusion" and injury to the right hepatic duct.

2.2. Prevention of injuries to hollow abdominal organs

Injuries to the duodenum and colon are serious complications occurring primarily in cases of severe adhesions, necessitating the application of specific safety techniques. In the presence of an indurated fibrotic fistulous tract the use of a laparoscopic stapler for simultaneous cutting and hermetic closure of the canal is recommended, which is a safer method than scissors and coagulation when the stomach or intestines are involved. When invisible microperforation of the duodenum is suspected, an intraoperative test with methylene blue through a nasogastric tube is applied, as staining of the field is a categorical sign of compromised integrity. For gentle separation of inflamed tissue planes, hydrodissection using the tip of the irrigation pump is used, with dissection mandatorily performed in close proximity to the wall of the gallbladder. In cases of severe deserosation or risk of perforation, placement of a wide drain (24–28 Ch) is appropriate, which allows early postoperative diagnosis of possible complications.

2.3. Bleeding control (Hemostasis)

Intraoperative bleeding from the liver bed or cystic artery can quickly worsen visualization and lead to technical errors; therefore, strict energy management is recommended using monopolar coagulation up to 45 W to avoid deep thermal damage to the parenchyma. Ideal dissection should be performed in the subserosal plane, where bleeding is minimal, although in cirrhosis or destructive cholecystitis this layer is often obliterated. When hemorrhage occurs, a sequential algorithm is applied: manual compression for 2–3 minutes, precise bipolar coagulation, or local hemostatic sealants. In cases of uncontrollable bleeding, patient safety requires immediate conversion.

2.4. Injury to the diaphragm and pleura

Although rare, right-sided injuries to the diaphragmatic dome and pleura are serious complications that can lead to immediate pneumothorax or hemopneumothorax as a result of mechanical trauma from extreme traction or thermal lesion. To prevent these conditions, the traction vector when pulling the gallbladder must be directed laterally and "outward" toward the abdominal wall, not only vertically upward, which ensures the necessary safe distance from the diaphragm. A key maneuver is the "dynamic grasper" technique, in which during the final separation of the gallbladder from the liver bed, the surgeon moves the instrument from the infundibulum toward the fundus (bottom), moving the organ away from the dangerous zone of the diaphragmatic dome. Particular attention is required when working with monopolar coagulation to avoid lateral thermal spread. Due to the fact that during artificial ventilation the lesion often remains latent, when an incident is suspected, patient safety requires immediate radiographic control and, if necessary, emergency pleural drainage.

2.5. Prevention of bile duct obstruction from residual stones or migrated stones

To ensure complete evacuation of stones from the biliary tree, intraoperative cholangiography (IOC) is established as the most powerful tool for prevention, as its routine application allows the discovery and removal of unsuspected stones in the common bile duct (CBD) during the same intervention. When choledocholithiasis is established, laparoscopic revision of the CBD is undertaken through extraction with a balloon catheter or choledochoscopy, and when wider access is needed, longitudinal

choledochotomy is applied for direct removal of the stones. As a critical safety measure, at any suspicion of impaired patency of the papilla or after traumatic revision of the ducts, placement of a Kehr drain is required, which ensures decompression and prevents bile leakage under pressure in the postoperative period.

2.6. Spillage of stones into the peritoneal cavity

Prevention through methods for preserving the integrity of the gallbladder, such as atraumatic graspers and careful manipulation with monopolar coagulation when dissecting the gallbladder from the liver bed. If stones are nevertheless spilled, they must be collected in Morrison's pouch with subsequent removal using an endobag, and if the stones are 7–8 mm in size, they can be aspirated.

2.7. Wound suppuration

Prophylaxis through use of an endobag, which prevents pathogens from entering the wound.

2.8. Postoperative hernias

Prevention through use of an endobag during gallbladder extraction and careful closure of the fascias with a single non-absorbable suture with a round needle.

Discussion

Analysis of contemporary data unambiguously shows that prevention of complications in laparoscopic cholecystectomy requires a transition from relying on individual skills to strict standardized protocols. Since visual misperception remains the leading cause of iatrogenic biliary lesions, routine achievement of the “Critical View of Safety” (CVS) is established as a mandatory contemporary standard. In parallel, the implementation of technologies for intraoperative navigation significantly reduces risk. While intraoperative cholangiography (IOC) is a classical method with proven efficacy, fluorescent ICG cholangiography offers an excellent non-invasive real-time alternative. Despite technological advances, however, clinical judgment and the human factor remain decisive. Effective team communication, recognition of high-risk situations, and timely application of “bail-out” strategies (such as subtotal cholecystectomy or conversion) should be viewed as a sign of surgical maturity, guaranteeing maximum patient safety.

Conclusion

Safety in laparoscopic cholecystectomy is a multifactorial process that begins with precise access to the abdominal cavity and ends with secure extraction of the organ. Minimizing intraoperative risks requires systematic training in anatomical navigation, non-alternative application of the CVS concept, and rational use of contemporary imaging methods (IOC and ICG). In the era of minimally invasive surgery, the supreme priority remains patient safety, which necessitates timely recognition of high-risk situations and readiness to transition to alternative and safe surgical strategies.

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Treatment options in cases of unilateral vocal cord paralysis: a literature review

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Introduction: Unilateral vocal cord paralysis is commonly idiopathic or iatrogenic and is associated with dysphonia, aspiration, ineffective cough, and dysphagia, significantly impacting quality of life. **Aim:** The aim of this study was to review the literature on treatment options of unilateral vocal cord paralysis and to present a contemporary algorithm for treatment planning. **Material and methods:** The material used for this review included peer-reviewed academic publications addressing the management of unilateral vocal cord paralysis. **Results and discussion:** Management of unilateral vocal cord paralysis is guided by etiology and time since onset, which both influence the likelihood of spontaneous recovery. Pharmacologic treatment has limited supporting evidence; however, early administration of nimodipine may accelerate neuronal regeneration. Medialization thyroplasty is the standard surgical treatment, which may be combined with arytenoid adduction in vertical vocal fold height mismatch. Laryngeal reinnervation restores vocal fold tone and bulk, with ansa-cervicalis-recurrent laryngeal nerve anastomosis offering phonation-synchronous activation and a low risk of synkinesis. Injection laryngoplasty is a minimally invasive intervention that improves glottic competence, voice quality and airway protection. In select injection materials, the effect may be temporary, suitable for early management. Autologous adipose tissue is increasingly favored for long-standing unilateral vocal fold paralysis due to its biocompatibility, and recent advancements in its processing may improve graft survival and augment long-term functional outcomes. **Conclusion:** Several treatment options exist in cases of unilateral vocal fold paralysis, and although most of them are not etiologic, they offer opportunities in ameliorating the cardinal symptoms of the condition.
***Keywords*:** *Unilateral vocal fold paralysis, Injection laryngoplasty*

Introduction

Unilateral vocal fold motion impairment has an estimated incidence of about 5 per 100,000 individuals in the general population. (1) When the underlying etiology is neurogenic, the terms *paresis* and *paralysis* are applied to denote partial and complete loss of mobility, respectively. These conditions are most commonly iatrogenic in origin, with reported incidences ranging from 2% to 10% following thyroid surgery and up to 21% after cervical spine procedures. (1, 2)

The second largest group of unilateral vocal fold paralysis (UVFP) cases is idiopathic UVFP, after exclusion of a wide range of potential causes. These include neurologic disorders such as Parkinson's disease, amyotrophic lateral sclerosis, Guillain-Barré syndrome, myasthenia gravis, and multiple sclerosis, as well as systemic and metabolic conditions including diabetes mellitus and hypokalemia. Additional considerations include Lyme disease, cerebrovascular events such as Wallenberg syndrome, and prior exposure to vinca alkaloids. (3)

The symptoms of UVFP commonly include dysphonia, dysphagia and decreased cough reflex. (4) Patients most often describe their voice as being hoarse, weak and breathy, sometimes also reporting vocal fatigue. (5) The swallowing dysfunction in patients with UVFP often presents with coughing or choking while eating, as well as food getting stuck in the throat and the need for increased attention during chewing and swallowing. This causes patients to employ compensatory mechanisms such as delayed eating, as well as increased attentional focus and changes in neck position during swallowing. (6)

The diagnostic workup of a new-onset UVFP begins with a complete head and neck examination. (7) Following, the essential

diagnostic tool is flexible laryngoscopy, which is the "reference" standard for examination of the larynx. Any asymmetry in the excursion of the vocal folds – one vocal cord relative to the other – represents a pathological process. (8) An emerging alternative method to assess movement disorders of the vocal folds is low-frequency ultrasound. (9) The flexible laryngoscope is also used to study the swallowing function, in addition to videoflouroscopy, allowing to assess the presence of penetration, aspiration and retained secretions. (10) Important supplemental diagnostic tests in cases of UVFP include computed tomography, magnetic resonance imaging and laryngeal electromyography. (7)

Aim

The aim of this study was to review the literature on treatment options of UVFP and to present a contemporary algorithm for treatment planning.

Methods

The material used for this review included peer-reviewed academic publications addressing the management of UVFP.

Results

Recurrent laryngeal nerve reanastomosis

Intraoperatively, if the recurrent laryngeal nerve (RLN) is interrupted, it is appropriate to immediately attempt a therapeutic resolution of the lesion. In defects less than 5 mm in length, RLN type end-to-end neurorrhaphy is applicable. While a risk of developing synkinesis exists, the advantages of the procedure outweigh it. [Milan] For larger defects, a free nerve graft from the greater auricular nerve, supraclavicular nerves or ansa cervicalis (ANSA) should be used, as it is imperative to avoid traction on the anastomosis. (11)

Drug therapy

Although historically numerous medications have been applied for treatment of UVFP, convincing evidence exists only for the therapeutic effect of the calcium antagonist nimodipine. It has been found to reduce calcium influx into the growth cone and to increase the rate of regeneration, increasing the rate of vocal fold movement recovery from 20% to 60% in patients with poor prognosis, as determined by laryngeal electromyography. (12, 13)

Vocal therapy

Vocal therapy exercises aimed at vocal fold adduction may be applied only in patients with maladaptive supraglottic compensation in order to increase supraglottic tone, without helping to restore vocal fold mobility itself. (7)

Medialization laryngoplasty

Isshiki developed laryngeal framework surgery in the 1970s, and medialization laryngoplasty (ML; Laryngeal Framework Surgery Type I) has since been considered the standard surgical treatment for UVFP. It is performed by placing an implant material, such as silicone or Gore-Tex, in the paraglottic space, displacing the paralyzed vocal fold medially. (14) The success rate of the procedure reaches 92%, with complications occurring in about 15% of cases. (15)

Medialization laryngoplasty may be combined with arytenoid adduction, hypopharyngoplasty and cricopharyngeal myotomy. The former two are applied in cases of a high vagal lesion with pronounced dysphagia. Arytenoid adduction is specifically employed in cases of a large glottic cleft with a vertical height mismatch in the posterior parts of the vocal folds during phonation. (16) The aim of the procedure is to reposition and stabilize the vocal process of the arytenoid of the immobile vocal fold. Arytenoid adduction is

not performed if the prognosis for RLN recovery is unknown. (17) Therefore, a laryngeal electromyography study confirming complete denervation of the vocal fold may aid in the clinical decision-making. (18) In hypopharyngoplasty, the excess mucosa of the flaccid, insensitive ipsilateral piriform sinus is plicated and the inferior constrictor is resuspended on the thyroid cartilage. (19, 20) As a result, the retention of secretions in the hypopharynx, which occurs in cases of a high vagal trauma, is shifted contralaterally, reducing the risk of aspiration. (7)

Medializing laryngoplasty may be further combined with pharyngoesophageal segment surgery to address coexisting dysphagia. The upper esophageal sphincter (UES) is a 3–5 cm high-pressure zone located between the hypopharynx and esophagus. It is formed by the inferior pharyngeal constrictor, cricopharyngeal muscle, and proximal cervical esophagus. The UES is tonically contracted at rest and receives dual innervation from the ipsilateral RLN and pharyngeal plexus. Injury to the vagus nerve or RLN may impair UES relaxation, leading to impaired bolus transit, retention of secretions in the hypopharynx and ascending pharyngeal weakness. Management options include cricopharyngeal dilatation, surgical myotomy or chemical denervation using botulinum toxin. (7)

Laryngeal reinnervation

Laryngeal reinnervation restores tone and volume of the denervated vocal fold, without improvement of mobility. (21) Among the various techniques currently described, ANSA-RLN anastomosis remains the most widely used approach. Reinnervation using the ANSA offers several advantages, including its accessibility within the neck and its favorable size match with the RLN. (4) In addition, the ANSA demonstrates endogenous discharge activity during phonation, contributing to more physiologic patterns of laryngeal activation. Because it is not a mixed nerve, there is also no risk of synkinesis development. (22) Clinical outcomes are generally superior when reinnervation is performed in younger patients and earlier in the course of the denervation process. (7)

Injection laryngoplasty

Injection laryngoplasty is a minimally invasive procedure that improves glottic competence, voice quality and airway protection. With certain injectable materials, the effect may be temporary, suitable for early treatment, when the prognosis of recovery is uncertain. Autologous fat is increasingly preferred for long-term treatment of UVFP due to its biocompatibility, and recent advances in processing techniques have been shown to improve graft survival and long-term functional outcomes. (23)

Discussion

The therapeutic approach in cases of UVFP is guided by the likelihood of spontaneous recovery. Functional recovery can refer to improvement in voice quality or recovery of vocal fold movement. Recovery of mobility does not guarantee improvement in voice. On the other hand, voice can improve without recovery of vocal movement, due to synkinetic reinnervation, which leads to a more medial position of the vocal fold and increased tone of the thyroarytenoid muscle. Recovery of voice without recovery of mobility is more common than recovery of movement. (24)

The potential for recovery of movement in UVFP depends on the etiology, time since onset and prognosis for recovery as assessed by laryngeal electromyography. Since the latter is not widely available, the main factor remains the time since onset, and a 12-month waiting period has long been applied. (7) However, newer studies have found that

most often, the recovery of UVFP movement occurs in the first 4–6 months and less often by the 9th month after onset, thereby shortening the recommended interval before definitive treatment. Another important consideration is the etiology, as idiopathic UVFP has a vocal fold motion recovery rate of 39%, while the recovery rate for iatrogenic UVFP is much higher, reaching 78%. (6, 24)

Treatment options which are definitive, such as medialization laryngoplasty and reinnervation procedures, are reserved for cases in which no recovery of vocal fold motion is expected. On the other hand, vocal and drug therapy, as well as injection laryngoplasty, with consideration of the injection material, are applicable in nearly all cases of UVFP.

Modifying factors when planning the optimal treatment strategy of UVFP include the presence of dysphagia and aspiration, as well as pulmonary comorbidities. Lesions of the RLN lead to dysfunction of multiple components of the swallowing mechanism, often leading penetration, aspiration, and reduced laryngeal clearance during swallowing, especially for liquids. (4, 25) In addition, reduced cough force due to the insufficient build-up of subglottic pressure further impairs lower airway protection. (26) The combination of an increased risk for aspiration and impaired cough reflex places patients at significantly increased risk for developing pneumonia. (4) Aspiration, that may worsen pulmonary function, especially in patients with pulmonary comorbidities, is an indication for immediate treatment. When recovery of motion is expected, injection laryngoplasty with a temporary injection material is the most suitable treatment modality; however, in other cases, laryngeal framework surgery is indicated.

Early treatment of UVFP is limited to the first 6 months after onset. (7) Early injection laryngoplasty has been found to reduce the risk of eventual medialization laryngoplasty. (7, 27) Due to the lack of strong evidence that early IL contributes to eventual functional recovery from UVFP, early IL should be encouraged because it improves function immediately, improving the patient's quality of life, and not because it contributes to eventual functional recovery from UVFP. (7)

Conclusion

Several treatment options exist in cases of unilateral vocal fold paralysis, and although most of them are not etiologic, they offer opportunities in ameliorating the cardinal symptoms of the condition.

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Application of total knee endoprosthesis with a mobile-bearing insert

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Total knee arthroplasty is the "gold standard" in treatment of advanced knee osteoarthritis. It is an effective procedure that leads to significant improvement in the quality of life of patients with severe degenerative disease of the joint. Knee prostheses are available with fixed-bearing and mobile-bearing designs. The main characteristic of the mobile-bearing insert is its ability to move relative to the tibial component, thereby preserving the natural biomechanics of the knee joint. The use of this type of insert provides greater congruency between the inlay and the femoral component, which in turn results in reduced and slower polyethylene wear. In the context of modern, fast-paced lifestyles and the increasing number of younger patients requiring knee arthroplasty, this insert design represents the method of choice. The mobile-bearing platform fully meets their high functional and physical demands.
Keywords: *osteoarthritis, total knee arthroplasty, mobile-bearing insert*

Introduction

Total knee arthroplasty (TKA) is one of the most common and successful surgical interventions in orthopedic surgery. Over the past decades, there have been significant improvements in the design and materials of implants, as well as in the instrumentation required for their implantation. Nevertheless, following knee endoprosthesis implantation, a certain percentage of patients – up to 10–23% according to various authors – report postoperative issues (1). The availability of various types of knee endoprostheses includes with and without posterior cruciate ligament retention, with mobile and fixed tibial platforms, single radius and multiple radius designs, as well as different implantation techniques – anatomic resection, measured resection, kinematic alignment which creates considerable confusion even among experienced knee surgeons. Which endoprosthesis is the best, and which implantation method yields optimal results? These are critical questions. The mobile tibial insert offers a range of theoretical advantages (2).

Objective

To share our experience with the application of a total knee endoprosthesis featuring a mobile tibial insert.

Materials and methods

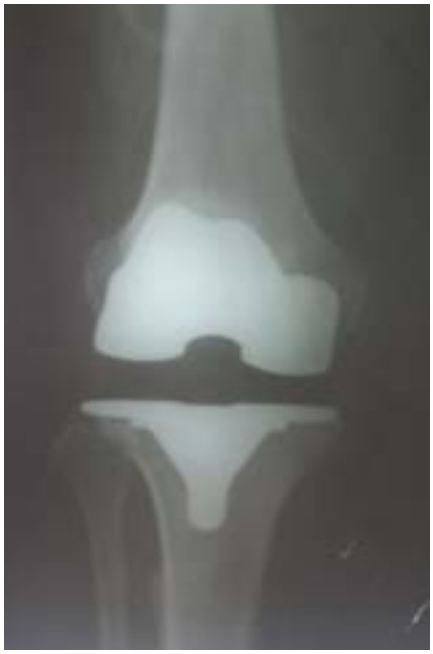
From October 2015 to December 2025, 453 patients – 350 women and 103 men, underwent surgery at the Clinic of Orthopedics and Traumatology of UMHAT "St. Georgi," Plovdiv. The mean patient age was 63 years (range: 54–78 years). All patients had previously undergone a series of conservative treatments prior to total knee arthroplasty (TKA): nonsteroidal anti-inflammatory drugs (NSAIDs), physiotherapy, knee bracing, weight reduction, intra-articular corticosteroids, chondroprotectors, platelet-rich plasma (PRP), and hyaluronic acid. Arthroscopic lavage and debridement of the affected knee was performed in 373 patients, providing temporary symptom relief. The Ahlbäck and Kellgren-Lawrence classifications were used to determine the degree of osteoarthritis (3). Preoperatively, all patients were at stage 3 or 4 (Graph. 1, 2).

Graph. 1.

Graph. 2.

Thirty-eight operated knees exhibited valgus deformity, while the remaining 415 showed varying degrees of varus deformity: 250 with mild deformity (≤ 10°), 170 with moderate deformity (10°–30°), and the remaining 33 patients with > 30° varus. Preoperative flexion and extension contractures of varying degrees were noted. In this series, the SAMO KNEE, Italy (Graph. 3, 4) was implanted in 320 patients, the FIRST, Symbios, Switzerland (Graph. 5) in 95 patients, and the SCORE II, Amplitude, France (Graph. 6, 7) in the remaining 38 patients. All three prosthesis models featured a mobile tibial platform, with components fixed using bone cement (cemented fixation).

Graph. 3.



Graph. 4.

Graph. 5.

A classic medial parapatellar approach was employed, enabling thorough debridement of all intra-articular structures and precise bone resections. During the procedure, proper positioning of the prosthetic components and adequate soft-tissue balancing of the joint were ensured.

Graph. 6.

Graph. 7.

Results

Patients were followed up for a period of one year. Functional outcomes were primarily assessed using the American Knee Society (AKS) Score (4). Regarding the AKS score, excellent results were reported in 290 patients, good in 110, satisfactory in 42, and poor in 11 patients. Postoperative joint pain resolved progressively. The need for analgesics ceased by postoperative day 3–4. The achieved range of motion in the prosthetic knee joint at the time of discharge varied from 0°–0°–115°.

During follow-up, the following complications were noted: superficial skin necrosis in 15 cases; in 10 patients, approximately one month post-discharge, rehospitalization was required due to flexion contracture of the operated knee. Serial manipulation of the prosthetic joint under general anesthesia was performed, followed by prolonged passive mobilization using a device. In 5 patients, radical synovectomy of the knee was performed six months postoperatively due to transient synovitis with effusion. Over the follow-up period, three cases of infectious complications were recorded, whereas deep vein thrombosis and/or pulmonary embolism were not observed.

Discussion

Successfully performed total knee arthroplasty (TKA) should meet three main criteria: pain-free function, stability, and mobility. Movements in the normal knee joint include not only flexion and extension, but also rotation and translation within the joint. In order to reduce the loading on the articulation between the femoral component and the polyethylene insert and to improve the kinematics of the prosthetic knee, Goodfellow and O'Connor (1976) developed a knee design in which there is a mobile articulation between the tibial component and the polyethylene (5, 6). The characteristics of the polyethylene design include a convex spherical femoral component and a tibial component that is flat on the inferior side and concave on the superior surface, matching the femoral convexity. The free motion of the polyethylene component is not dictated by the joint design itself, but rather by the capsuloligamentous apparatus of the knee. Initially, the mobile-platform prosthesis was conceived as an unicompartmental device. Total knee arthroplasty with a mobile bearing was first introduced by Buechel and Pappas in 1977. Some designs of mobile total knee bearings allow only rotational freedom of the polyethylene insert, whereas others also permit anteroposterior translation. Normal knee kinematics include posterior roll-back of the femur relative to the tibia and internal axial rotation during flexion (7). In vivo fluoroscopic studies of various fixed-bearing knee designs have demonstrated a reduction of normal femoral roll-back and axial rotation, as well as paradoxical reverse axial rotation and abnormal anteroposterior femoral translation during deep flexion. Normal rotation is of paramount importance for physiological patellofemoral articulation. D'Lima et al. show that TKA with a fixed bearing significantly alters tibiofemoral articulation, whereas a mobile platform more closely replicates physiological kinematics (8, 9). In this design, the primary rotation occurs at the lower articulation – between the insert and the tibial component. This feature reduces shear forces between the components and at the bone–cement interface and theoretically increases the longevity of the prosthesis. According to Bottlang et al., these shear forces are reduced by up to 68–73% in the mobile articulation (10). Polyethylene wear is substantially decreased in prostheses with a mobile tibial platform (11). Several studies have

demonstrated long-term survivorship of mobile-bearing knee arthroplasty comparable to that achieved with fixed-platform TKA (12, 13, 14).

Conclusion

The results obtained after the use of a mobile tibial platform in total knee arthroplasty are similar to those reported for fixed-platform TKA. However, in younger and more active patients, the mobile concept offers a theoretical advantage, related primarily to the reduced contact stress between prosthetic components under load and to the decreased polyethylene wear.

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Current international recommendations for cervical cancer screening: a literature review

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In 2025, leading international organizations issued updated global recommendations placing primary human papillomavirus (HPV) testing as the new gold standard for cervical cancer screening. This model replaces the traditional Papanicolaou (Pap) test as the primary screening method. The strategies of the World Health Organization (WHO), the U.S. Preventive Services Task Force (USPSTF), the American Cancer Society (ACS), and the International Agency for Research on Cancer (IARC), applicable within the European context, differ mainly with regard to the age at which screening should commence, the prioritization of HPV testing, and the organization of population-based versus opportunistic screening programs. A review of the literature demonstrates the effectiveness of both screening methods and highlights that HPV testing has higher sensitivity for the detection of precancerous lesions compared with cytology-based screening.

Keywords: *cervical cancer, current international recommendations, screening*

Introduction

Cervical cancer remains a significant global public health concern despite being largely preventable [1, 2]. According to recent GLOBOCAN estimates, more than 600,000 new cases are diagnosed annually worldwide, with over 340,000 deaths each year [1, 2]. More than 80% of these deaths occur in low- and middle-income countries [1, 4]. Persistent infection with high-risk human papillomavirus (hrHPV), particularly types 16 and 18, represents the principal etiological factor [1, 3].

Over the past decade, a major paradigm shift has occurred in cervical cancer screening, transitioning from cytology-based programs to primary HPV-based screening [3]. Leading international bodies – including the World Health Organization (WHO), the European Commission, the U.S. Preventive Services Task Force (USPSTF), the American Cancer Society (ACS), the United Kingdom National Health Service (NHS), and the Australian National Cervical Screening Program (NCSP) – increasingly endorse HPV testing as the preferred screening modality [3].

Despite this convergence, differences remain regarding initiation age, screening intervals, upper age limits, and the role of cytology and self-collection [3, 5]. A comparative evaluation of these recommendations is essential within the broader framework of the global strategy for cervical cancer elimination [3].

Aim

The aim of this literature review is to analyze and compare current international cervical cancer screening recommendations (2025–2026), with specific focus on:

1. Screening model (primary HPV versus cytology-based approaches);
2. Age of initiation and cessation;
3. Screening intervals following a negative result;
4. The role of cytology and self-collection;
5. Global epidemiological trends and survival projections.

Materials and methods

A narrative literature review was conducted based on official international guidelines, policy documents, and epidemiological data sources. Recommendations from WHO, the European Commission (EC-CvC and Council Recommendation), USPSTF, ACS, NHS, and the Australian NCSP were analyzed, together with global statistics from IARC (GLOBOCAN) and SEER.

Documents published or updated between 2020 and 2026 were included, with emphasis on the most recent guideline versions. A comparative qualitative synthesis was performed to identify similarities, differences, and emerging trends in international screening strategies.

Results

A comparative analysis of contemporary international cervical cancer screening guidelines demonstrates substantial convergence toward primary high-risk HPV (hrHPV) testing as the preferred screening modality.

The World Health Organization recommends primary HPV testing within a structured screen–triage–treat framework, initiating screening at 25 years with intervals of 3–5 years depending on resource setting [6]. European policy documents similarly endorse organized population-based primary HPV screening and explicitly discourage cytology or co-testing as primary modalities within organized programs [10, 11].

In the United States, the USPSTF draft recommendation maintains cytology every three years for women aged 21–29 years, while preferring primary HPV testing every five years for women aged 30–65 years [7]. The American Cancer Society recommends initiation at 25 years with primary HPV testing every five years as the preferred strategy [8], including formal endorsement of self-collected HPV samples [9, 18]. HRSA has incorporated patient-collected HPV testing within preventive service coverage policies [17].

The United Kingdom has implemented a national HPV-primary screening program for women aged 25–64 years, with extended five-year intervals following a negative HPV result [12–14]. Australia applies a comparable primary HPV model with partial genotyping, screening from 25 to 74 years, including structured exit testing between 70–74 years and systematic integration of self-collection pathways [15, 16].

Across most organized programs, cytology now functions primarily as a triage method in HPV-positive cases rather than as a standalone screening test [10–12, 15].

Discussion

The reviewed guidelines reflect a decisive global shift from cytology-based screening toward molecular risk stratification through HPV testing. The higher sensitivity and negative predictive value of HPV assays justify the extension of screening intervals and enable risk-adapted screening algorithms [6, 8, 12].

Despite increasing harmonization, certain policy differences remain. The United States retains greater flexibility by allowing cytology-based screening from age 21 [7], whereas WHO, ACS, the UK, and Australia initiate screening at 25 years [6, 8, 12, 15]. These differences likely reflect historical screening infrastructures and transitional phases in implementation.

Upper age limits also vary. Most programs conclude routine screening at 64–65 years [7, 8, 12], while Australia extends screening to 74 years within a structured exit-testing framework [15]. This approach reflects modeling data and long-term programmatic evaluation.

The integration of self-collection represents one of the most significant contemporary developments in screening strategy. Its systematic implementation in Australia [16], formal endorsement by ACS [9], and incorporation into U.S. preventive policy [17] demonstrate growing institutional confidence in this approach. European guidance similarly encourages strategies aimed at increasing participation within organized systems [10].

Collectively, these developments align with the broader international strategy for cervical cancer elimination centered on vaccination, high screening uptake, and timely management of precancerous lesions [6].

Conclusion

International cervical cancer screening strategies are increasingly aligned around primary HPV-based testing, reflecting a fundamental transformation in preventive care. Molecular screening has replaced cytology as the central tool for early detection in most organized programs, allowing longer intervals and more individualized risk assessment.

Remaining differences in initiation age, cessation age, and implementation models illustrate that screening policy is shaped not only by scientific evidence but also by healthcare structure and national priorities. The expansion of validated self-collection strategies and structured population-based programs represents a meaningful step toward improving access and equity.

Future progress will depend on integrating vaccination status into screening algorithms, strengthening follow-up systems, and ensuring sustainable program implementation across diverse healthcare environments. With continued refinement and coordinated public health efforts, cervical cancer control is moving steadily from early detection toward realistic elimination.

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Advances in gynecological laparoscopy: applications of artificial intelligence – literature review

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Introduction: Laparoscopy has become a clinically important component in gynaecology as it provides better patient outcomes than open surgery. While being dependent on the high precision and skill of the surgeon, the use of artificial intelligence is explored as a supportive tool in intraoperative guidance and surgical training. **Aim:** The aim of this literature review is to summarise the current literature on the applications of artificial intelligence in laparoscopic gynecology, benefits, limitations, challenges, and future prospects. **Materials and methods:** Literature was selected through studies focusing on applications in gynecologic laparoscopy. **Results and discussion:** The reviewed literature suggests that artificial intelligence can be applied in preoperative planning, intraoperative decision-making and surgical training, improving surgical outcomes in gynecological laparoscopy. Its application in surgical training allows for an increased number of gynecological laparoscopic experiences, overcoming current ethical limitations in teaching gynecological laparoscopy. However, challenges exist, such as the reluctance to adopt artificial intelligence into clinical practice due to a lack of familiarity, ethical and legal issues concerning patient consent and data privacy, and cost and resource constraints. Limitations of artificial intelligence in gynaecology include limited datasets, which may limit the model's performance in further cases. **Conclusion:** Overall, artificial intelligence shows potential to improve gynecological laparoscopic surgery through enhanced visualisation, surgical education and objective skill assessment, leading to improved patient outcomes. However, challenges exist to fully integrate artificial intelligence models in the clinical setting.
Keywords: *gynecological laparoscopy, artificial intelligence, intraoperative guidance, surgical training*

Introduction

Over the past decades, laparoscopic surgery has evolved into a fundamental component of modern gynecological practice, largely due to its association with reduced surgical trauma, shorter hospital stay, and improved postoperative recovery. Despite these advantages, laparoscopic procedures remain highly dependent on the surgeon's experience, psychomotor skills, and intraoperative decision-making, which introduces variability in surgical performance and outcomes.

Recent technological advances have positioned artificial intelligence (AI) as an emerging adjunct in laparoscopic surgery. Contemporary studies demonstrate that AI systems are capable of real-time recognition of anatomical structures and surgical instruments, enabling enhanced intraoperative orientation and safety [1, 2]. Furthermore, automated identification of surgical phases and workflow analysis has been shown to support procedural standardization and optimize operative efficiency [3, 4].

In parallel, AI-based models for objective assessment of surgical skills are gaining increasing attention, offering reproducible and unbiased evaluation of technical performance, which is particularly relevant for surgical training and certification [5–8]. Additionally, the integration of AI into robotic-assisted laparoscopy represents an important step toward precision surgery, with potential benefits in terms of accuracy, ergonomics, and clinical outcomes [4, 9].

Taken together, these developments highlight the growing role of artificial intelligence as a supportive tool in gynecologic laparoscopy, with implications extending from intraoperative guidance to education and quality assurance. The aim of this review is to summarize current evidence regarding AI applications in gynecological

laparoscopy and to evaluate their clinical relevance and future perspectives [10, 11].

Aim

The aim of this literature review is to summarize the current evidence on the applications of artificial intelligence in laparoscopic gynecology, including its benefits, limitations, challenges, and future prospects.

Materials and methods

This review was conducted through a targeted search of the PubMed database for articles published within the past three years (2023–2025), focusing on the applications of artificial intelligence in gynecologic laparoscopy. The search strategy included combinations of the following keywords: “gynecologic laparoscopy”, “artificial intelligence”, “deep learning”, “machine learning”, “image recognition”, “instrument detection”, “surgical phase recognition”, “skill assessment”, and “robotic surgery”.

Results and discussion

Analysis of the included studies indicates that artificial intelligence is progressively being incorporated into gynecologic laparoscopic care, including preoperative planning, intraoperative decision-making, and surgical training. Advances in computer vision have enabled real-time detection of surgical instruments and anatomical structures, thereby enhancing intraoperative orientation and potentially improving procedural safety [1, 2]. In addition, AI-based systems capable of recognizing surgical phases and analyzing workflow have shown promise in optimizing operative efficiency and supporting standardized surgical performance [3, 4].

A particularly dynamic area of development is the application of AI in surgical training. Several studies report that machine learning models can objectively assess technical skills based on motion tracking, image analysis, and procedural metrics, offering reproducible and unbiased evaluation of surgical performance [5–8]. This has important implications for surgical education, as AI-assisted training platforms may allow trainees to accumulate a greater number of laparoscopic experiences in a controlled and ethically acceptable environment, partially overcoming current limitations associated with patient-based learning.

Furthermore, the integration of AI into robotic-assisted laparoscopy represents an important step toward precision surgery. By combining enhanced visualization with algorithm-driven guidance, such systems may improve surgical accuracy and ergonomics while supporting real-time decision-making [4, 9]. At a broader level, recent reviews emphasize the growing role of AI as a supportive tool in gynecologic surgery, with potential benefits extending beyond the operating room to include quality assurance and performance monitoring [10, 11].

Despite these promising developments, several challenges remain. A notable barrier is the reluctance to adopt AI technologies into routine clinical practice, often driven by limited familiarity and trust among clinicians. Ethical and legal concerns related to patient consent, data security, and accountability also represent significant obstacles to widespread implementation. In addition, the high cost of advanced AI systems and the need for substantial computational resources may limit accessibility, particularly in resource-constrained settings.

From a methodological perspective, one of the key limitations identified across studies is the reliance on relatively small and highly selected datasets. This may restrict the generalizability of AI models and reduce their performance when applied to more diverse clinical scenarios.

Conclusion

Artificial intelligence is redefining the landscape of gynecologic laparoscopic surgery by enhancing visualization, supporting surgical training, and enabling objective evaluation of technical performance. Its integration into surgical practice holds the promise of greater precision, improved standardization, and ultimately better patient outcomes.

At the same time, the path toward full clinical implementation remains challenging. Barriers related to acceptance, ethical and legal considerations, and resource availability must be carefully addressed. Ensuring robust validation, transparency, and clinical reliability will be essential for the safe adoption of these technologies.

In this context, artificial intelligence should be viewed not as a replacement for surgical expertise, but as a powerful complementary tool – one that has the potential to transform gynecologic laparoscopy from an experience-dependent discipline into a more standardized, data-driven, and consistently reproducible practice.

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Diagnostic and therapeutic challenges in managing fluid-electrolyte disturbances following supratentorial craniopharyngioma surgery: a case report

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Background: A supratentorial craniopharyngioma is a rare, benign brain tumour that grows above the tentorium cerebelli. While more commonly found in paediatric patients, they can present at all ages. The neurosurgical treatment for tumours of sellar and suprasellar origin frequently results in sodium and fluid disturbances. **Case Presentation**: This report presents the case of a 10-year-old boy born after an uneventful pregnancy and delivery, with normal neuropsychological development. The patient presented with progressive visual impairment, headaches and gastrointestinal disturbances upon admission. Computed tomography demonstrated a supratentorial mass consistent with craniopharyngioma. Following craniotomy and ventriculoperitoneal shunt placement, there was a development of a complex course of early postoperative hypernatremia and hyponatremia fluctuations. On day 1 following surgery, the patient experienced symptoms associated with Central Diabetes Insipidus, namely hypernatremia and polyuria. Management required the provision of desmopressin and hypotonic fluids. From day 5 post-surgery, the patient developed severe hyponatremia, polyuria, natriuresis and altered consciousness consistent with Cerebral Salt Wasting Syndrome. The patient was treated with hypertonic fluids and fludrocortisone. These complications are rare and can lead to a high mortality rate, as they require opposing treatment strategies. **Conclusion:** This case highlights the difficulties of managing postoperative sodium and fluid disturbances following removal of a supratentorial craniopharyngioma, particularly in distinguishing between biphasic and triphasic patterns of Central Diabetes Insipidus, Syndrome of Inappropriate Antidiuretic Hormone Secretion and Cerebral Salt Wasting Syndrome. Timely recognition and vigilant observation in the postoperative period are critical to prevent progressive neurological morbidity and improve patient prognosis.

Keywords: *craniopharyngioma, hyponatremia, hypernatremia, biphasic, craniotomy*

Introduction

Craniopharyngioma is a rare, benign but locally aggressive epithelial tumour that originates from the embryonic remnants of Rathke's pouch, typically located in the sellar or suprasellar region. (1) They account for 1.2–4% of all primary intracranial tumours and a significant portion in paediatric patients. (1) In children, it frequently presents with headaches, visual disturbances and hypothalamic-pituitary dysfunction. Although surgical intervention remains an important treatment option, the proximity of the tumour to the pituitary stalk and hypothalamus exacerbates the threat of postoperative endocrine and electrolyte complications. (4) We aim to present a case with multiple postoperative endocrine and electrolyte syndrome considerations, highlighting the challenge of a triphasic approach to diagnosing and treating such patients.

Case presentation

A 10-year-old boy with an unremarkable perinatal history and normal neuropsychological development was diagnosed at the age of 8 with myopia and regularly monitored by an ophthalmologist. 6 months post-diagnosis, during a regular examination, he developed progressive bilateral visual disturbances, presenting with asymmetrical changes in visual acuity. These changes were accompanied by recurrent headaches, nausea and vomiting, with neuroimaging revealing a craniopharyngioma. Following this diagnosis, the patient underwent surgical resection. On admission, there were no clinical signs of meningeal irritation, and

neurological examination revealed left central facial nerve palsy with preserved muscle tone. Static and intention tremor and right horizontal fine nystagmus without diplopia were evident. Additionally, bilateral papilledema with blurred optic disc margins was observed with cranial computed tomography, demonstrating findings suggestive of craniopharyngioma.

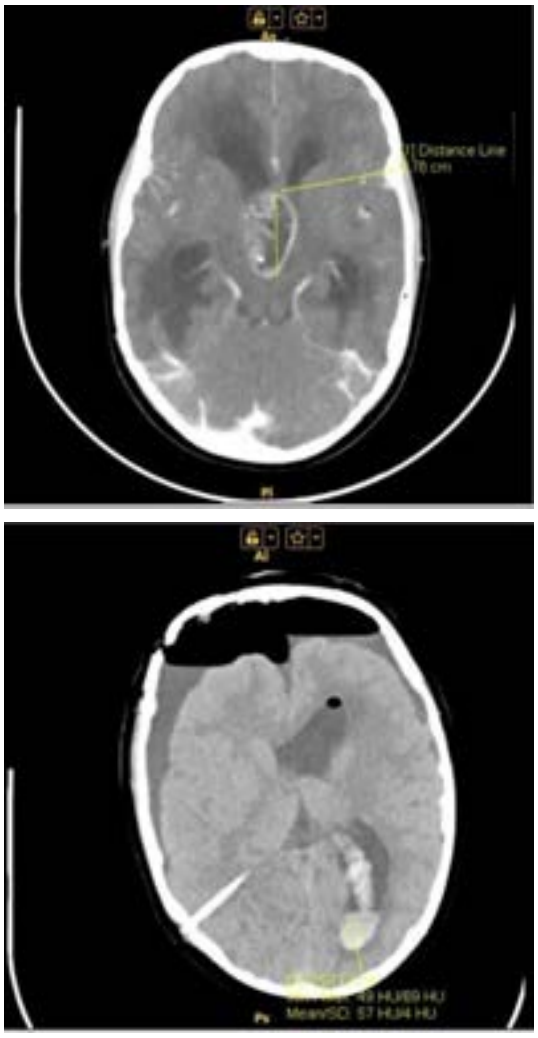

Figure 1 & 2 show first CT scan on the 29.04.2021 & 24 hours after operation on the 21.05.2021

Neurosurgical intervention took place on 29th May for right parieto-occipital craniotomy and ventriculoperitoneal shunt implantation. Immediately after surgery, the serum sodium was initially normal, then increased to 150 mmol/l on the first postoperative day, indicating postoperative central diabetes insipidus. By the fifth day, significant hyponatremia (120 mmol/l) developed, accompanied by polyuria, natriuresis, and altered consciousness requiring reintubation. Despite continuous 0.9% NaCl infusion, hyponatremia persisted at 129 mmol/l on days 27–28, which then normalised to 143 mmol/l on 28th May. The patient was extubated on 1st June with a GCS of 9–10 and neurological status impaired. The patient presented with reduced muscle tone and eyes opening to painful stimuli. There was also absent visual tracking and voluntary activity, while oculocephalic and pharyngeal reflexes remained preserved.

Discussion

Postoperative disturbances in sodium and water balance are well-recognised complications following surgery for craniopharyngioma due to injury or dysfunction of the hypothalamic-pituitary axis. Studies have shown that patients with craniopharyngiomas often present with pituitary hormone deficiencies in approximately 28.6%, with central diabetes insipidus in children. (3) Hyponatremia in this case was the central diagnostic challenge. The disturbances may manifest as a triphasic sequence of ADH deficiency, transient syndrome of inappropriate antidiuretic hormone (SIADH) and possible diabetes insipidus. The primary differential diagnosis included SIADH and cerebral salt wasting syndrome (CSWS), which are well-established causes of hyponatremia in postoperative neurosurgery. SIADH is defined by the excessive secretion of antidiuretic hormone, resulting in water retention. Conversely, CSWS is sodium loss, causing hyponatremia, polyuria and natriuresis. Distinction is critical as both disturbances require fundamentally different treatment strategies. In the first phase of the triphasic

response, surgical damage to the pituitary pathways (via pituitary stalk transection) leads to a decrease in ADH secretion and subsequent ADH deficiency. This manifests as hypernatremic dehydration (3), hence symptoms of polydipsia, polyuria and hypoosmolar urine are shown. Monitoring of body weight, diuresis, serum sodium, urine specific gravity, and osmolality should be performed to recognise this condition. In the second phase, syndrome of inadequate ADH is observed, where oliguria and hyponatremia are presented, with this phase typically lasting anywhere from 2 to 14 days. (3) Free water is commonly held during this phase, and therefore treatment is reflected through fluid restriction. The third phase is not universally observed; however, it is characterised by a long-term course (months to years), sometimes lifelong, central diabetes insipidus due to depleted ADH. Symptoms such as polyuria, high levels of serum sodium and bouts of dehydration are persistent. (3) The prognosis may persist indefinitely.

Conclusion

While surgical approaches exist for treating craniopharyngiomas, a triphasic treatment strategy – aligned with the disease's characteristic triphasic course – offers the most effective approach for patient management. This case illustrates the complexity of postoperative dysnatremia following craniopharyngioma resection due to the overlapping of SIADH and CSWS. Since these conditions demand opposed fluid management strategies, early clinical recognition, precise volume assessment, and frequent biochemical monitoring are paramount. Implementing these practices is critical to navigating the volatile triphasic response, thereby preventing severe neurological complications and reducing associated patient mortality.

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II. Biomedical Session

The diagnostic value of breast MRI for post-augmentation complications: a case report

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Breast augmentation is one of the most performed aesthetic procedures in the world. Despite this fact, it is often associated with complications, such as implant ruptures and silicone leakage. Magnetic resonance imaging (MRI) is the most sensitive imaging modality for breast diagnostics and is central for evaluating implant integrity and related complications. Here we present the case of a 47-year-old patient with previous augmentation mammoplasty, who was referred for MRI after routine ultrasound indicated suspicion of a large tumor-like mass in the lower medial quadrant of the right breast. The MRI shows symmetrical breast parenchyma with age-appropriate involution bilaterally. Both breast implants appear with signs of rupture: with complete capsular rupture on the left, and intracapsular rupture of the right implant. Native sequences reveal the presence of an additional substance bilaterally, appearing hypointense on silicone-sensitive sequences and hyperintense on silicone-suppression sequences, forming a conglomerate in the lower media quadrant of the right breast, corresponding to the detected suspicious lesion. Such particles were also identified in the axillary areas bilaterally. No pathological contrast enhancement or diffusion restriction was observed, arguing against malignancy. Axillary lymph nodes were of borderline size with preserved fatty hilum. Breast MRI has a crucial role in complex post-augmentation scenarios, differentiating between implant related complications and neoplastic processes, therefore preventing misdiagnosis and unnecessary invasive procedures.

Keywords: *MRI, diagnostic, implant rupture, complications*

Introduction

Breast augmentation is frequently encountered in breast-imaging practice and radiologists must differentiate normal implant appearances from clinically important complications. Silicone gel implants may rupture silently, and physical examination alone may not identify many silicone implant ruptures which is why periodic ultrasound or MRI surveillance is recommended, with MRI advised for symptomatic patients or equivocal ultrasound findings [1, 2].

MRI has diagnostic value after augmentation because implant-dedicated protocols combine anatomic sequences with silicone-sensitive and silicone-suppressed imaging. These sequences allow differentiation of intracapsular rupture, extracapsular silicone and mimics such as seroma, gel bleed, inflammatory change or non-implant filler material. When a post-augmentation mass-like abnormality is detected by ultrasound, contrast-enhanced MRI and diffusion-weighted imaging (DWI) add functional information that helps distinguish benign non-enhancing material from a malignant lesion [3–5].

Case presentation

A 47-year-old woman with a history of bilateral augmentation mammoplasty underwent routine breast ultrasound during follow-up. She had no acute breast symptoms. Ultrasound revealed a large, ill-defined, tumor-like formation in the lower medial quadrant of the right breast, raising concern for malignancy. Because the finding was unclear and both breasts contained implants, the patient was referred for breast MRI.

MRI was performed using T1-weighted non-fat-suppressed imaging for anatomy and implant morphology; STIR and silicone-specific sequences for silicone detection and

water/silicone signal separation; DWI with apparent diffusion coefficient (ADC) mapping and dynamic post-contrast imaging with parametric enhancement maps. This approach was selected because standard morphologic images alone may be insufficient to distinguish implant rupture, extracapsular material and enhancing neoplasm in augmented breasts.

MRI demonstrated bilaterally symmetrical breast parenchyma with age-appropriate involution. Both implants showed rupture-related changes. The left implant had complete capsular rupture with implant contents confined by the surrounding fibrous capsule. The right implant showed intracapsular rupture with silicone between layers of the implant shell. In addition to implant-related findings, multiple small bilateral breast-parenchymal deposits were present. A larger conglomerate was identified in the lower medial quadrant of the right breast, corresponding to the lesion suspected on ultrasound (Fig. 1).

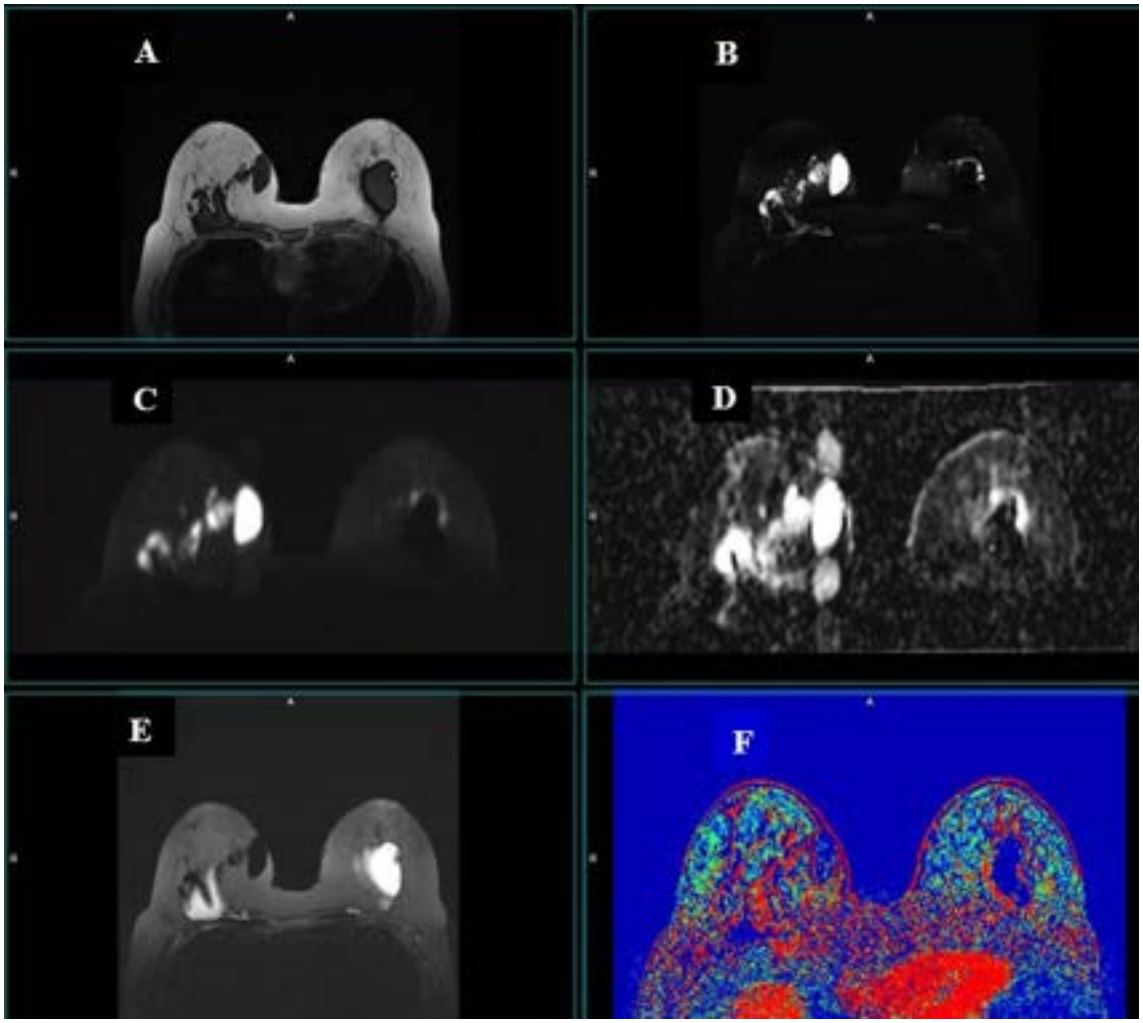

Figure 1. Multiparametric axial breast MRI sequence. Sequences used:
A. T1-weighted image without fat suppression – hypointensity
B. STIR for silicone – suppresses fat and helps highlight silicone – hyperintensity
C. DWI (hyperintensity) and
D. ADC map (also hyperintensity) – no restricted diffusion
E. STIR with water suppression – hypointensity
F. PEI-map (post-contrast/parametric enhancement) – no vascular enhancement.

The material demonstrated fluid-like signal characteristics: it was hypointense on T1-weighted imaging and hyperintense on STIR/water-based sequences with behavior not fully explained by the right-sided intracapsular rupture. DWI showed no true restriction, with no corresponding low ADC values and dynamic contrast-enhanced imaging showed no suspicious enhancement. Similar particles were also detected in both axillary regions. The axillary lymph nodes were borderline in size but retained preserved fatty hila. The combined pattern supports a benign post-augmentation complication rather than malignancy. After MRI review, additional history was obtained and the patient disclosed prior injection of gel filler into the breasts for enlargement. The final interpretation was bilateral implant rupture with additional injected hydrophilic gel/filler material mimicking a mass-like lesion on ultrasound, without MRI evidence of malignancy.

Discussion

This case demonstrates three important diagnostic contributions of breast MRI. First, MRI accurately classified implant integrity. Intracapsular rupture is diagnosed when silicone remains within the fibrous capsule, but the implant shell is disrupted; extracapsular rupture is diagnosed when silicone extends beyond the capsule. In the present case, MRI separated left complete capsular rupture from right intracapsular rupture and showed that the right lower-medial mass-like finding was not simply explained by extracapsular leakage from the right implant.

Second, silicone-selective and silicone-suppressed sequences were essential for material characterization. Dedicated MRI sequences exploit differences between fat, water and silicone signals, increasing confidence in detecting small-volume silicone or silicone-like deposits and in mapping their distribution. This was particularly important because injected filler and leaked silicone can both create echogenic or mass-like abnormalities on ultrasound. The diffuse bilateral and axillary distribution, combined with the signal pattern and the patient's later disclosure, favored injected hydrophilic gel/filler material as the explanation for the suspicious right-sided conglomerate.

Third, MRI helped exclude malignancy. Dynamic contrast-enhanced breast MRI evaluates vascularity and enhancement kinetics, while DWI/ADC evaluates water mobility and can support differentiation between benign and malignant breast lesions [3–5]. The absence of suspicious enhancement and absence of true diffusion restriction in this case argued against a malignant mass.

The case also emphasizes the value of complete clinical history. Patients may not initially report prior cosmetic filler injection, and undocumented injections can mimic implant-related disease or neoplasm. MRI cannot replace careful history-taking, but it can direct the clinician toward the correct differential diagnosis by showing whether a lesion enhances, restricts diffusion, follows silicone distribution or behaves like non-implant fluid/filler material.

Conclusion

Breast MRI is a decisive problem-solving modality in complex post-augmentation imaging. In this patient, it confirmed bilateral implant rupture, characterized a right breast tumor-like lesion as non-malignant injected filler material rather than cancer and demonstrated no suspicious enhancement or diffusion restriction. A structured multiparametric MRI protocol can therefore prevent misdiagnosis and may avoid unnecessary

invasive procedures in patients with post-augmentation complications.

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From CT to MRI: comprehensive characterization of a cerebellopontine angle schwannoma – a case report

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Cerebellopontine angle (CPA) tumors are common posterior fossa lesions and may cause severe deficits when enlarging masses compress the brainstem or obstruct cerebrospinal fluid (CSF) pathways. Vestibular schwannoma (VS) is the most common tumor of the cerebellopontine angle, accounting for approximately 80–90%. Diagnostic imaging is crucial for definitive and differential diagnosis. We present a case highlighting the added value of MRI after CT detection. A 73-year-old female patient with slowly progressive unilateral hearing impairment, intermittent tinnitus and mild steadiness underwent contrast-enhanced CT of the head, which revealed a sharply marginated, oval, extra-axial left CPA mass measuring 4.2×3.0 cm, predominantly isodense to brain parenchyma, with small calcified components, and showing intense, near-homogeneous enhancement. There was marked mass effect, including compression of the left cerebellar hemisphere (with mild edema) and the pons with rightward displacement, fourth-ventricle compression, and dilatation of the lateral and third ventricles consistent with obstructive hydrocephalus. Subsequent MRI (T2 TSE/T2 TIRM, T1, DWI, SWI, 3D TOF, post-contrast T1) confirmed the extra-axial CPA lesion, demonstrated relatively homogeneous T2 hyperintensity with strong enhancement, showed no restricted diffusion, and identified multiple susceptibility foci on SWI compatible with hemosiderin. 3D TOF demonstrated preserved Circle of Willis anatomy. The imaging findings of our patient supported the diagnosis of CPA schwannoma and provided comprehensive pre-treatment assessment, underscoring MRI as the cornerstone modality beyond CT for characterization, differential diagnosis, and management planning.
Keywords: *Vestibular schwannoma, Cerebellopontine angle, Brainstem compression*

Introduction

Vestibular schwannoma is a benign, slowly growing intracranial tumor, typically well-circumscribed by a capsule. It arises from the Schwann cells of the vestibular portion of the vestibulocochlear nerve and is the most frequent lesion in the cerebellopontine angle (CPA). It most commonly occurs sporadically in patients aged between 40 and 60 years, whereas cases associated with Neurofibromatosis Type 2 tend to present at an early age [1] [2].

The symptoms of the schwannoma may not be noticeable for many years as the average growth rate of the lesion is about 1 mm per year and is related to the compression of the nerves – vestibulocochlear, facial and trigeminal [2] [3]. With progressive enlargement of the lesion, involvement of the pons and cerebellum may also occur, along with obstructive hydrocephalus [4].

On non-contrast CT, small vestibular schwannomas may be difficult to detect, particularly when limited to the internal auditory canal. CT is, however, useful for evaluating bone changes, calcifications, hydrocephalus, and mass effect [1].

Our case shows that there was no evidence of widening of the internal acoustic canal, a rare finding observed in only approximately 20% of cases [1].

Case presentation

We present a 73-year-old female patient with slowly progressive unilateral hearing impairment, intermittent tinnitus and mild gait unsteadiness – symptoms suggestive of vestibulocochlear nerve involvement. There was no family history of Neurofibromatosis Type 2, nor intracranial tumors. Laboratory tests were within normal limits.

Contrast-enhanced CT of the head (arterial and venous phases) revealed an extra-axial oval-shaped tumor lesion with well-defined margins, located in the left cerebellopontine angle and measuring 4.2 × 3.0 cm.

The mass appeared isodense to the parenchyma of the brain on non-contrast imaging and intense, nearly homogeneous contrast enhancement was observed on post-contrast images. There was marked dilatation of the lateral and third ventricles, consistent with obstructive hydrocephalus (Fig. 1).

On T2-weighted and T2-TIRM sequences of MRI, the lesion demonstrated relatively homogeneous hyperintensity. Following contrast administration, intense enhancement was observed, without diffusion restriction. Furthermore, a significant mass effect on the left cerebellar hemisphere was noted, associated with white matter edema, as well as compression and rightward displacement of the pons and fourth ventricle (Fig. 2).

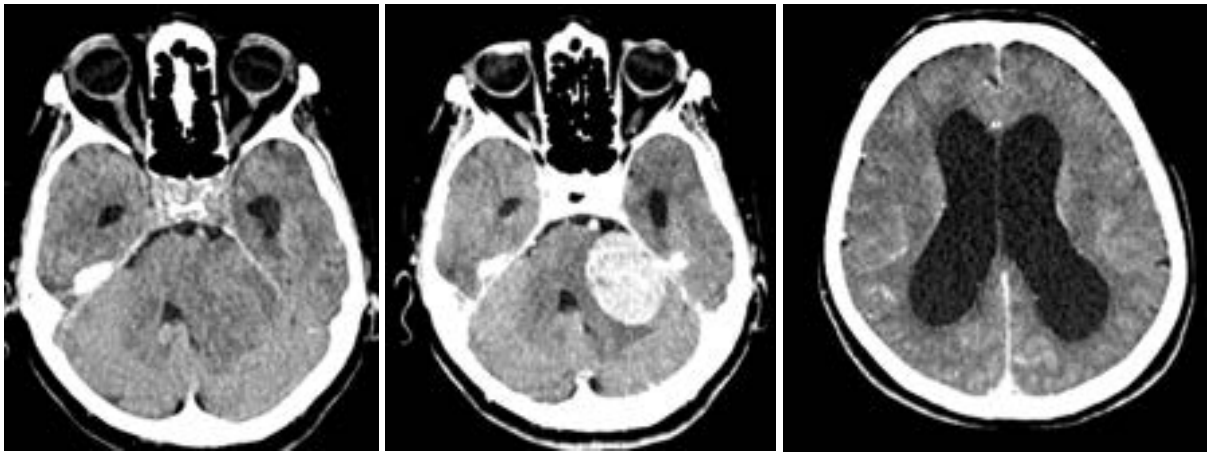

Figure 1. CT findings. (A) Non-contrast axial CT demonstrates a left cerebellopontine angle mass. (B) Contrast-enhanced CT shows avid enhancement of the lesion. (C) Axial CT demonstrates dilatation of the lateral ventricles, consistent with obstructive hydrocephalus

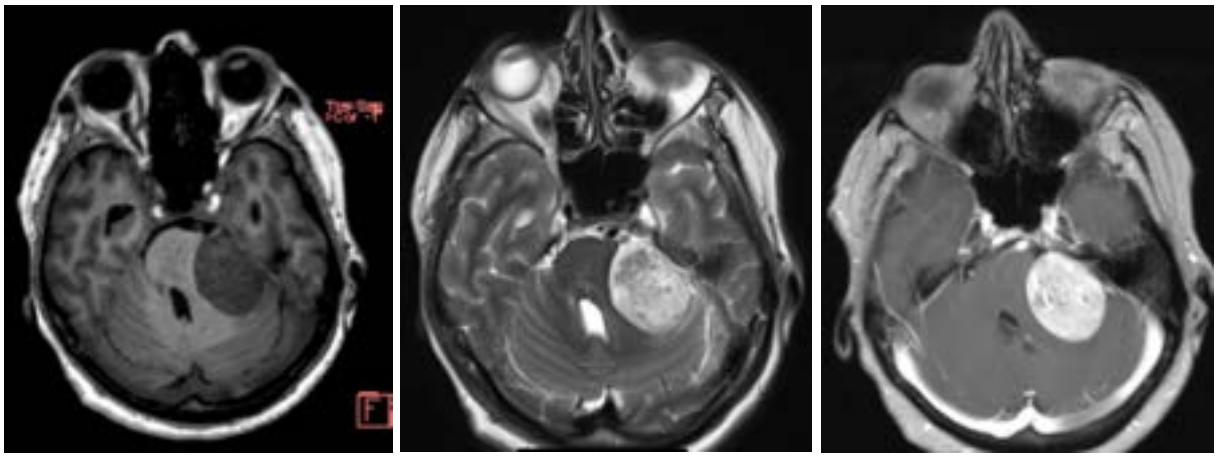

Figure 2. MRI findings. (A) Axial T1-weighted image. (B) Axial T2-weighted image demonstrates a hyperintense left CPA lesion. (C) Post-contrast T1-weighted image shows avid enhancement of the lesion.

Discussion

Our case highlights the roles of CT and MRI in the diagnostic evaluation of vestibular schwannoma. CT is useful as an initial imaging method, particularly for demonstrating a large cerebellopontine angle mass, its mass effect, compression of the fourth ventricle, associated obstructive hydrocephalus, calcifications, and possible bony changes of the internal auditory canal [7–9]. However, CT has limited soft-tissue contrast and is less accurate than MRI for defining the exact tumor extent, its relationship to the brainstem and cranial nerves, and the differential diagnosis of cerebellopontine angle lesions [7, 8]. MRI is the imaging modality of choice for suspected vestibular schwannoma because it provides superior tissue characterization and detailed anatomical assessment of the cerebellopontine angle and internal auditory canal [7, 8, 10]. Contrast-enhanced T1-weighted MRI is considered the gold standard for the initial imaging diagnosis of vestibular schwannoma, while high-resolution T2-weighted/CISS sequences provide complementary information regarding the internal auditory canal, cranial nerves, CSF spaces, and tumor margins [10, 11]. In the present case, MRI confirmed the extra-axial CPA location of the lesion, demonstrated strong enhancement, absence of diffusion restriction, and susceptibility foci compatible with hemosiderin, supporting the diagnosis of vestibular schwannoma. MRI is also essential for distinguishing vestibular schwannoma from other CPA lesions, particularly meningioma and epidermoid cyst [7–10]. Meningioma may show a broad dural base, dural tail, calcification, and hyperostosis, whereas epidermoid cysts typically show marked diffusion restriction and lack avid solid enhancement [7, 9]. Susceptibility-weighted imaging may further assist in differentiating schwannomas from meningiomas, as intratumoral blooming foci related to microhemorrhage are more frequently observed in schwannomas [12].

Conclusion

CT was essential for the initial detection of the cerebellopontine angle mass and for demonstrating mass effect and obstructive hydrocephalus. MRI provided superior soft-tissue characterization, better assessment of tumor extent, and greater confidence in the diagnosis of vestibular schwannoma. Therefore, CT has an important role in the initial diagnostic work-up, whereas MRI remains the imaging modality of choice for definitive characterization, differential diagnosis, and preoperative planning.

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Blue nevus – a case report and literature review

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Blue nevus is a benign, macroscopically well-circumscribed melanocytic lesion that can be present at birth or develop later in life. It is more common in females from Asian populations than the Caucasian population, where it is rare. This case report aims to present the clinical features and histopathological findings of a blue nevus located on the hand of a female patient, highlighting the pivotal role of histological evaluation in achieving an accurate diagnosis. A 50-year-old woman was admitted to the hospital with complaints of progressively increasing pain in the left antebrachium. Clinical examination revealed a pigmented lesion measuring 1 cm in diameter with a grayish-black coloration. The lesion was surgically excised. The specimen was processed using standard histopathological techniques and subsequently evaluated by light microscopy. The histological results were consistent with a benign blue nevus. To substantiate the diagnosis and rule out an incipient malignant transformation, the section was decolorized and subjected to immunohistochemical analysis for p16 expression, which was positive as well as Ki-67 which demonstrated a low proliferative index of approximately 5%. In conclusion, this case highlights the importance of thorough histopathological evaluation in the assessment of clinically atypical, pigmented lesions, especially when accompanied by progressive symptoms. Careful microscopic examination and appropriate immunohistochemical analysis are essential to exclude early malignant transformation and to guide appropriate clinical decision-making.

Introduction

Blue nevi are benign melanocytic lesions that typically present as solitary, blue-to-black nodules or papules. Pathogenetically, these lesions result from the incomplete migration of primordial melanocytes to the epidermis during embryonic development, resulting in their arrest within the dermis (1). The classic blue appearance is governed by the Tyndall effect, where shorter wavelengths of light are scattered by dermal collagen while longer wavelengths are absorbed by deep-seated melanin (2). Clinically, blue nevi are categorized into two primary variants: common and cellular. The common variant is typically found on the head, neck, or extremities and is usually smaller than 1 cm, whereas the cellular variant is often larger and frequently localized to the sacral region (3). Despite these subtypes, blue nevi are traditionally defined as asymptomatic and stable (4). We report a case that deviates from this classic clinical presentation. The differential diagnosis for a painful pigmented lesion includes dermatofibroma, glomus tumor, or malignant melanoma; only limited cases, such as a recently reported tender scalp lesion, exist in current literature (5). A blue nevus presenting with localized pain is exceptionally rare, challenging initial clinical suspicion and underscoring the importance of histopathological evaluation.

Case presentation

A 50-year-old woman sought medical attention for a symptomatic skin lesion on her antebrachium (forearm). The patient's primary complaint was progressively increasing localised pain, representing an atypical clinical feature. Physical examination revealed a solitary, well-demarcated nodule

with deep pigmentation. To address the discomfort and exclude malignancy, the lesion was surgically excised under local anesthesia. The specimen was processed using standard histopathological techniques for light microscopy and immunohistochemical analysis (p16 and Ki-67). Histologically, hyperpigmented spindle-shaped nevus cells were concentrated below the epidermal layer. Deeper dermal evaluation demonstrated a hyperpigmented zone of nevus cells surrounding hair follicles, which is a characteristic finding. These cells were situated between elastic fibers within the reticular dermis and extended into the hypodermis. Exhibits demonstrated spindle patterns and focal hyperpigmentation. To exclude cellular atypia, slides were decolorized. No significant atypia was noted despite deep penetration. Immunohistochemistry confirmed p16 positivity with no loss of expression. The Ki-67 proliferative index was approximately 5%, further confirming the benign nature of the lesion.

Discussion

Blue nevus was first recognized by Tieche in 1906, having previously been labeled chromatophoroma or melanofibroma (6). There is historically significant difficulty in distinguishing these lesions from Mongolian spots (6). Prevalence rates vary globally, with Asian populations showing a higher prevalence (3–5%) compared to Caucasians (1–2%) (7). While 1 in 3000 neonates present with blue nevi at birth, they typically develop during adolescence (7). Histologically, blue nevi are defined by dermal localization and hyperpigmentation (8). Our case exhibited both features but showed unusual extension into the hypodermis. Although melanoma developing from a blue nevus is an extremely rare complication, the patient's localized pain contradicted the typical stable clinical profile (8). A 2018 case report described limb pain and cyanosis associated with blue nevi, which in that instance related to cholesterol microembolism (9). Furthermore, syndromes such as Blue Rubber Bleb Nevus (BRBN) may present with venous malformations and pain extending into subcutaneous tissues, though BRBN typically involves systemic organ involvement and abdominal symptoms (10, 11). Notably, compared to blue nevi, melanoma exhibits a vast range of immunohistochemical markers such as S-100, HMB-45, MART-1, tyrosinase, and MITF, which facilitate rapid diagnosis (12). The potential for malignancy remains a grave concern; a study of 12 malignant blue nevus cases reported high rates of recurrence, metastasis, and mortality, even in patients with no family history of melanoma (13). This underscores the impetus for managing blue nevi carefully, particularly when they present with atypical symptoms like pain.

Conclusion

In summary, blue nevus must be accurately identified regarding its resemblance to melanoma. While typically asymptomatic, the atypical presentation of pain in this case highlights the necessity of systematic clinical and histological assessment. Given the rarity of malignant transformation and the potential for poor prognosis if unrecognized, practitioners must prioritize thorough diagnostic evaluation. Enhanced study of these lesions is required to refine clinical management and prognostic accuracy when atypical symptoms occur.

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Chronic osteomyelitis mimicking a malignant bone tumour: a case study

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An important diagnostic challenge in chronic osteomyelitis is in distinguishing between infection and malignancy, due to their similar aggressive radiological features. This obscurity often leads to confusion between these two entities, needing careful differential diagnosis. This report presents a case of chronic osteomyelitis of the lower limb with imaging findings highly suggestive of malignancy and emphasises the important role of histopathological verification in differential diagnosis. A 62-year-old female with a history of multiple surgical interventions involving the right lower leg underwent magnetic resonance imaging due to a progressively enlarging soft tissue mass. Imaging findings were followed by surgical biopsy and a histopathological analysis. Magnetic resonance imaging showed a large heterogeneous lesion with extensive soft tissue involvement, cortical disruption of the tibia and fibula, areas of necrosis and haemorrhagic foci, diffusion restriction and heterogeneous contrast enhancement. These features were radiologically concerning for a malignant process, including sarcomatous neoplasms. Histopathological examination showed changes consistent with chronic osteomyelitis without evidence of malignancy. This case highlights the substantial overlap in imaging characteristics between chronic infection and malignant bone tumours, especially following prior surgical interventions. Chronic osteomyelitis should be considered an important differential diagnosis in patients who present with radiologically aggressive bone and soft tissue lesions. Correlation between imaging findings, clinical history, and histopathological assessment is essential to establish an accurate diagnosis and to prevent unnecessary or inappropriate oncological treatment.

Introduction

Chronic osteomyelitis (COM) is a serious musculoskeletal infection of the bone characterised by inflammation, soft-tissue changes, and progressive bone destruction. Population-based data indicate that osteomyelitis incidence ranged from about 11 to 24 cases per 100,000 person-years over several decades, with an increasing burden in older patients and males. (1) Chronic osteomyelitis may be classified using systems such as the Cierny–Mader classification, which considers anatomical and host factors, further influencing clinical presentation and imaging findings. Clinical presentation may be indolent, however radiological findings can be aggressive, especially in patients with long-standing diseases or repeated history or trauma. In these settings, imaging can resemble that of primary malignant bone or soft tissue tumours

Magnetic resonance imaging (MRI) plays a crucial role in the assessment of suspected musculoskeletal pathology due to its excellent soft-tissue contrast and sensitivity to bone marrow abnormalities. Features such as cortical destruction, soft tissue masses, necrosis, heterogeneous contrast enhancement, and diffusion restriction are non-specific, as they can be observed in both chronic infection and malignancy. The overlap can lead to significant diagnostic ambiguity, resulting in diagnostic uncertainty and prompting an extensive oncological workup. (2)

This report emphasises the critical role of histopathological evaluation, culture, and sensitivity testing, remaining the definitive methods for diagnosis. Accurate diagnosis through a multidisciplinary approach is essential for avoiding misclassification and inappropriate treatment, as the management strategies and prognostic implications differ significantly. (3)

Case presentation

We present a 62-year-old female with a background history of multiple prior surgical interventions of the right lower limb with no known personal history of malignancy. She presented with chronic pain, progressive localised swelling and a gradual increase in size of the right lower leg. She reported no associated weight loss or night sweats. There was raised suspicion of an underlying malignancy due to progression and local aggressiveness.

Magnetic resonance imaging of the lower leg was conducted with native and post-contrast sequences in three planes. It demonstrated a large, irregular, heterogeneous soft tissue lesion involving the lateral and ventral aspects of the limb, extending from the proximal metaphysis to the distal diaphysis of the tibia, measuring approximately 19 cm in craniocaudal length.

The lesion appeared hypointense on T1-weighted images and heterogenous hyperintense on T2-weighted images, with heterogenous contrast enhancement. Restricted diffusion, areas of necrosis and small foci of haemorrhage were identified along with overlying skin ulceration.

There was significant osseous involvement, including cortical disruption and destruction of the tibia and fibula and marrow oedema. These imaging features were highly suggestive of an aggressive malignant process, prompting histopathological evaluation.

Due to the extensive local involvement, the patient underwent surgical intervention in the form of limb amputation. Histopathological examination of the resected specimen demonstrated features consistent with chronic osteomyelitis, with no evidence of malignancy, as communicated by the treating team.

Discussion

This case showcases an important example of chronic osteomyelitis presenting with radiological features highly suggestive of malignancy. The overall imaging appearance closely mimicked that of a high-grade sarcomatous process. This highlights the potential for chronic infection to manifest as a pseudotumoral lesion, posing a significant diagnostic challenge even with advanced imaging modalities. (4)

Several radiological features raised suspicion of malignancy. The lesion demonstrated extensive soft-tissue involvement, a large, heterogeneous mass, and cortical destruction affecting both the tibia and the fibula. Areas of necrosis and haemorrhage further supported this impression, as did the presence of diffusion restriction, which is typically seen in hypercellular malignant tumours. However, these imaging characteristics are not specific. In chronic osteomyelitis, persistent inflammation, necrotic bone (sequestrum) and granulation tissue can show a similar radiological appearance. Diffusion restriction in this context may result from increased cellularity and the presence of purulent material, thereby mimicking malignancy.

A significant contributing factor in this case is the patient's history of multiple prior surgical interventions involving the affected limb. It is well recognised that previous surgical procedures are predisposing factors for chronic osteomyelitis, due to impairment of vascularity, potential for bacterial colonisation and disruption of normal tissue planes. These changes can promote a chronic low-grade infectious process that progresses over time. It may not present with classic systemic features of infection. (5) In this situation, the aggressive radiological appearance observed in our patient may be misleading, as the underlying pathology shows chronic

inflammatory processes and reparative processes as opposed to a neoplastic transformation. Recognition of such predisposing factors is therefore essential in guiding appropriate differential diagnosis and preventing premature attribution to malignancy.

Misdiagnosis of chronic osteomyelitis as a malignant process can lead to patients being subjected to unnecessary invasive procedures, extensive biopsies or consideration of radical surgical resection. This is often accompanied by an avoidable oncological workup, such as staging CT or PET imaging, which further increases healthcare utilisation. In rare cases, suspicion of malignancy may lead to inappropriate initiation of oncological therapies. The psychological impact of a presumed cancer diagnosis would contribute to the patient's distress and anxiety. Collectively, these factors underscore the importance of accurate diagnosis in minimising avoidable harm while enhancing clinical outcomes (6)

Histopathological evaluation remains the gold standard in differentiating chronic osteomyelitis from bone tumours. In the present case, biopsy confirmed chronic osteomyelitis and excluded malignancy despite highly suspicious imaging findings. Malignant lesions are characterised by atypical mesenchymal cells with increased mitotic activity, nuclear pleomorphism, hyperchromasia, and an infiltrative growth pattern. In contrast, osteomyelitis typically demonstrates necrotic bone, inflammatory infiltrates, and granulation tissue. In longstanding cases, chronic osteomyelitis carries a recognised risk of malignant transformation, such as squamous cell carcinoma, which may arise from chronic sinus tracts, warranting careful assessment.

Conclusion

Chronic osteomyelitis can closely resemble malignant bone tumours both clinically and radiologically, particularly in patients with predisposing factors such as prior surgical intervention. This case emphasises the importance of recognising the limitations of radiological assessment in isolation and underscores the need to maintain a broad differential diagnosis for musculoskeletal lesions. Early biopsy and histopathological evaluation remain essential for establishing an accurate diagnosis and preventing unnecessary or inappropriate oncological management. Continued vigilance and multidisciplinary collaboration are necessary to optimise patient outcomes as diagnostic strategies evolve.

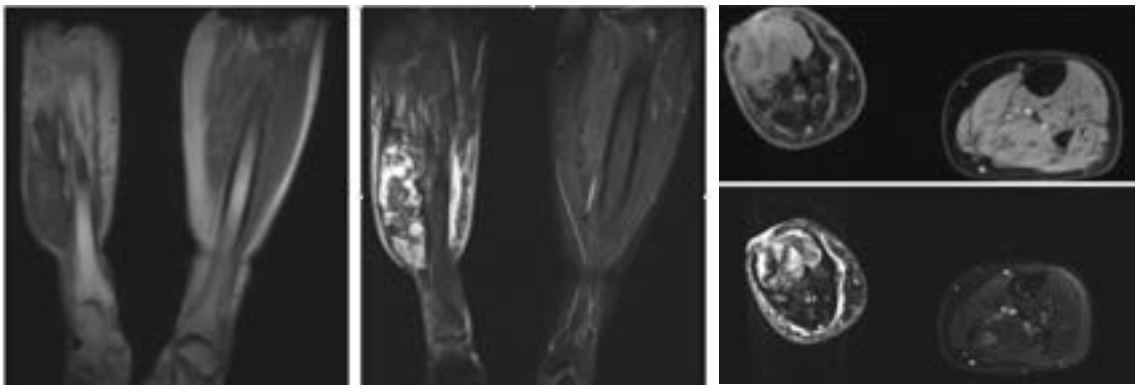

Figure (A): Coronal T1-weighted MRI showing a large hypointense soft tissue lesion with cortical destruction of the tibia and fibula. **Figure (B):** Coronal T2/STIR MRI demonstrating a heterogeneous hyperintense lesion with surrounding soft tissue and marrow oedema. **Figure (C):** Transverse T1-weighted MRI showing extensive soft tissue involvement and cortical disruption. **Figure (D):** Transverse T2/STIR MRI demonstrating heterogeneous high signal intensity with necrotic and inflammatory changes, mimicking malignancy.

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The gut-endocrine axis: microbiome regulation of insulin resistance, cortisol signalling, oestrogen metabolism, and appetite

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Introduction: The gut microbiome functions as a dynamic endocrine interface modulating metabolic, neuroendocrine, and reproductive signalling through microbial metabolites, immune–neuroendocrine crosstalk, and host–microbe interactions. Despite growing interest, evidence across endocrine domains remains heterogeneous. **Aim:** To synthesise mechanistic, experimental, and human evidence linking the gut microbiome to insulin resistance, hypothalamic–pituitary–adrenal (HPA) axis activity, oestrogen metabolism, and appetite regulation, and to identify key sources of inconsistency and translational gaps. **Materials and methods:** A narrative synthesis of in-vitro studies, animal models, human observational cohorts, and emerging interventional trials was performed, with findings interpreted by endocrine domain and mechanistic pathway. **Results and discussion:** Microbiome-derived short-chain fatty acids, bile acids, and inflammatory mediators modulate insulin sensitivity and glucose homeostasis, though human causality remains debated. Experimental data strongly support microbiome involvement in HPA-axis regulation and cortisol signalling, while clinical associations vary with stress exposure, microbial diversity, and host susceptibility. The estrobolome regulates oestrogen deconjugation and enterohepatic recycling, linking gut composition to reproductive and hormone-dependent disorders, yet population-level findings remain inconsistent. Appetite and energy balance are governed by gut–brain signalling via enteroendocrine hormones, neural pathways, and microbial metabolites, with marked interindividual variability in response to a or probiotic modulation. **Conclusion:** The gut microbiome is a central but incompletely defined regulator of endocrine homeostasis. While mechanistic plausibility is strong, translation is limited by heterogeneity and unresolved bidirectionality. Future research should prioritise longitudinal, life-course, and intervention-based designs integrating microbiome profiling with endocrine phenotyping.
Keywords: *gut microbiome; endocrine axis; insulin resistance; HPA axis; cortisol; estrobolome; appetite; short-chain fatty acids; gut-brain axis; microbiome-endocrine crosstalk*

1. Introduction

1.1. The gut microbiome as a dynamic endocrine interface

The human gut microbiome comprises a complex and dynamic ecosystem of bacteria, archaea, viruses, and fungi that collectively contribute to metabolic, immunological, and neuroendocrine homeostasis (1). Beyond its traditional role in digestion, emerging evidence suggests that the gut microbiot functions as a bidirectional endocrine interface through the production and modulation of bioactive metabolites, including short-chain fatty acids (SCFAs), secondary bile acids, neurotransmitter precursors, and inflammatory mediators. These microbial-derived signals interact with enteroendocrine cells, immune pathways, and neuroendocrine axes to influence host glucose metabolism, appetite regulation, stress responsiveness, and sex hormone homeostasis (2).

Simultaneously, host endocrine factors such as cortisol, insulin, and sex steroids can alter microbial composition and intestinal barrier integrity, highlighting the reciprocal nature of host–microbiome communication. Collectively, these findings position the gut microbiome as an important integrative regulator linking metabolic, immune, and endocrine physiology.

1.2. Microbial metabolites, immune–neuroendocrine crosstalk, and host–microbe interactions

Microbial metabolites constitute key mediators of communication between the gut microbiome and host endocrine systems. Short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, regulate

intestinal barrier integrity, glucose homeostasis, and enteroendocrine hormone secretion through interactions with G-protein-coupled receptors such as GPR41 and GPR43 (3).

In parallel, microbial modification of bile acids influences metabolic and inflammatory pathways via activation of farnesoid X receptor (FXR) and Takeda G-protein receptor 5 (TGR5). The gut microbiota also contributes to immune–neuroendocrine crosstalk by modulating cytokine production, intestinal permeability, and hypothalamic–pituitary–adrenal (HPA) axis activity, thereby linking inflammation with endocrine and stress-related signalling (4).

Furthermore, bidirectional host–microbe interactions are shaped by diet, medications, stress exposure, and hormonal status, all of which can alter microbial diversity and metabolic function (5). These interconnected pathways collectively support the concept of the gut microbiome as a dynamic signalling network influencing both local gastrointestinal physiology and systemic endocrine regulation.

1.3. Rationale and scope of the review

Growing interest in the gut microbiome has led to increasing recognition of its potential role in endocrine regulation; however, evidence across metabolic, neuroendocrine, and reproductive domains remains heterogeneous and, at times, contradictory (6). While experimental studies have demonstrated mechanistic links between microbial metabolites, immune signalling, and hormonal pathways, translation into consistent human clinical outcomes remains limited by methodological variability, population heterogeneity, and unresolved issues of causality and bidirectionality.

Most existing literature focuses on individual endocrine disorders in isolation, with comparatively limited integration of shared mechanistic pathways across endocrine systems. Therefore, this narrative review aims to synthesise current mechanistic, experimental, and clinical evidence regarding microbiome involvement in insulin resistance, hypothalamic–pituitary–adrenal axis activity, oestrogen metabolism, and appetite regulation, while critically examining translational limitations, methodological challenges, and future directions for precision endocrine medicine.

2. Overview of the gut–endocrine axis: key mechanistic pathways

2.1. Short-chain fatty acids as signalling molecules

Short-chain fatty acids (SCFAs), principally acetate, propionate, and butyrate, are among the most extensively studied microbial metabolites linking the gut microbiome to host endocrine and metabolic regulation. Produced through bacterial fermentation of non-digestible dietary carbohydrates within the colon, SCFAs exert both local and systemic effects through activation of G-protein-coupled receptors including free fatty acid receptor 2 (FFAR2/GPR43) and free fatty acid receptor 3 (FFAR3/GPR41) (7). Beyond serving as an energy substrate for colonocytes, particularly in the case of butyrate, SCFAs contribute to maintenance of intestinal barrier integrity, modulation of inflammatory responses, and regulation of glucose and lipid metabolism (figure 1).

Experimental studies further demonstrate that SCFAs stimulate enteroendocrine secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), thereby influencing insulin sensitivity, satiety signalling, and energy homeostasis (7–8). In addition, SCFAs may exert epigenetic effects through histone deacetylase inhibition, linking microbial metabolism with host gene expression and endocrine signalling pathways (8) (figure 1).

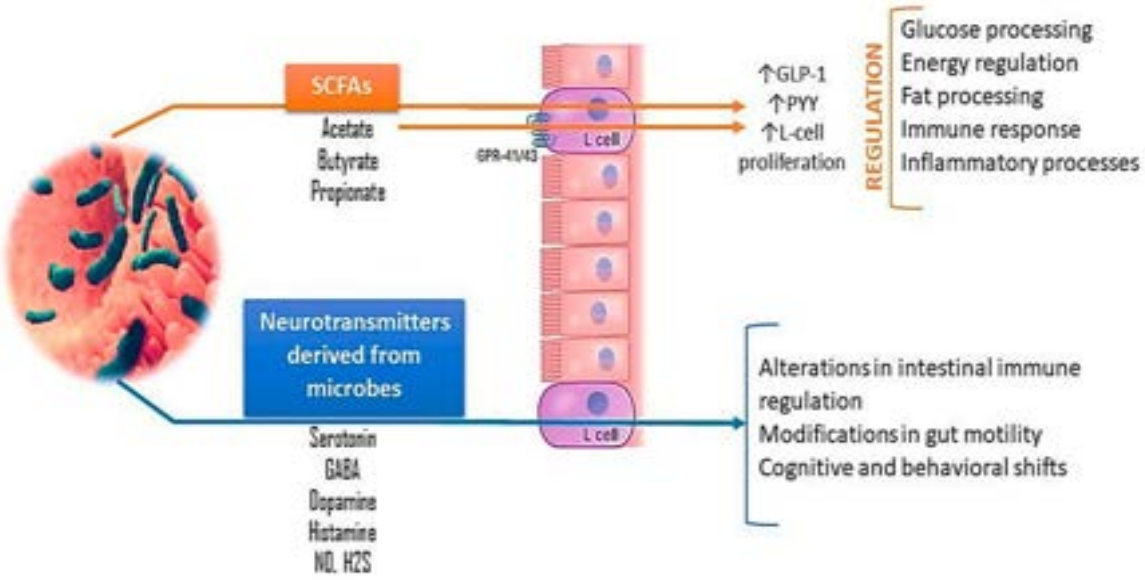

Figure 1. Gut microbiota influence metabolism, hormones, and disease through SCFAs and neurotransmitters.

Although mechanistic evidence supporting SCFA-mediated endocrine regulation is substantial, translation into consistent human clinical outcomes remains limited by interindividual variability in diet, microbial composition, and methodological heterogeneity across microbiome studies.

2.2. Bile acid metabolism and receptor signalling (FXR, TGR5)

The gut microbiome plays a critical role in bile acid metabolism by transforming primary bile acids synthesised in the liver into secondary bile acids through microbial deconjugation and biotransformation reactions. These bile acid metabolites function not only in lipid absorption but also as endocrine signalling molecules capable of modulating systemic metabolic homeostasis (9). Central to these effects are the farnesoid X receptor (FXR) and Takeda Gprotein-coupled receptor 5 (TGR5), which mediate interactions between microbial bile acid metabolism and host endocrine pathways.

FXR signalling influences hepatic gluconeogenesis, lipid metabolism, and insulin sensitivity, while TGR5 activation has been associated with enhanced glucagon-like peptide-1 secretion, increased energy expenditure, and improved glucose regulation (9). Through these receptormediated pathways, alterations in gut microbial composition may contribute to metabolic disorders including obesity, insulin resistance, and metabolic dysfunction-associated steatotic liver disease (9–10).

Nevertheless, considerable variability exists between experimental and clinical findings, and the precise contribution of microbiome-derived bile acid signalling to endocrine disease pathogenesis in humans remains incompletely defined.

2.3. Inflammatory mediators and endocrine crosstalk

Inflammatory signalling represents a major mechanistic interface through which the gut microbiome influences endocrine physiology. Disruption of intestinal microbial homeostasis may impair epithelial barrier integrity and increase intestinal permeability, facilitating translocation of microbial products such as lipopolysaccharide (LPS) into systemic circulation (11). This phenomenon,

commonly termed metabolic endotoxaemia, promotes chronic lowgrade inflammation through activation of toll-like receptor 4 (TLR4), nuclear factor-kappa B (NF-κB), and downstream pro-inflammatory cytokines including tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).

Persistent inflammatory activation has been implicated in insulin receptor dysfunction, adipose tissue inflammation, hypothalamic–pituitary–adrenal (HPA) axis dysregulation, and altered reproductive hormone signalling. Furthermore, inflammatory pathways may both influence and be influenced by endocrine dysfunction, highlighting the bidirectional and self-reinforcing nature of microbiome–endocrine interactions (12).

While mechanistic and animal studies strongly support a role for inflammatory mediators in linking gut dysbiosis to endocrine disease, interpretation of human data remains complicated by confounding factors including obesity, diet, medication exposure, and underlying metabolic comorbidities.

2.4. Enteroendocrine cells as transducers of microbial signals

Enteroendocrine cells (EECs) serve as specialised sensory transducers that convert luminal microbial and nutritional signals into endocrine and neuroendocrine responses (13). Distributed throughout the gastrointestinal epithelium, EECs detect microbial metabolites including SCFAs and secondary bile acids through an array of nutrient-sensing receptors, subsequently regulating secretion of hormones such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), serotonin, and ghrelin (13–14). These hormones influence multiple physiological processes including appetite regulation, glucose homeostasis, gastrointestinal motility, and energy balance.

In addition to endocrine signalling, EECs communicate with the central nervous system through vagal afferent pathways, forming an important component of the gut–brain axis. Emerging evidence suggests that alterations in microbial composition and metabolite production may therefore modulate both metabolic and behavioural responses via enteroendocrine signalling networks (13–15).

However, substantial interindividual variability in microbial responsiveness and endocrine outcomes continues to limit the translation of these findings into consistent therapeutic strategies targeting the gut–endocrine axis.

3. Microbiome regulation of insulin resistance and glucose homeostasis

3.1. SCFA-mediated insulin sensitisation (GPR41/43 Signalling)

Short-chain fatty acids (SCFAs), particularly acetate, propionate, and butyrate, have emerged as important mediators linking the gut microbiome to insulin sensitivity and glucose homeostasis. Generated through microbial fermentation of dietary fibre within the colon, SCFAs exert endocrine and metabolic effects primarily through activation of free fatty acid receptors FFAR2 (GPR43) and FFAR3 (GPR41), which are expressed in enteroendocrine cells, adipose tissue, and immune cells (16). Activation of these receptors has been associated with enhanced secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), improved insulin responsiveness, modulation of adipocyte metabolism, and regulation of hepatic glucose production. In particular, butyrate contributes to maintenance of intestinal epithelial integrity, thereby reducing microbial translocation and inflammation-associated insulin resistance.

Experimental studies further suggest that SCFAs may influence host metabolism

through epigenetic regulation via histone deacetylase inhibition, linking microbial activity with endocrine gene expression and energy homeostasis (16–17).

Although animal and mechanistic studies strongly support a beneficial role for SCFAs in metabolic regulation, translation into consistent human outcomes remains variable due to differences in diet, microbial composition, obesity status, and methodological heterogeneity across clinical studies.

3.2. Microbiome-driven inflammation and insulin receptor interference

Chronic low-grade inflammation is considered a major pathway through which gut microbial dysregulation may contribute to insulin resistance and impaired glucose metabolism. Alterations in gut microbial composition can disrupt intestinal barrier integrity, increasing epithelial permeability and facilitating translocation of bacterial components such as lipopolysaccharide (LPS) into systemic circulation (18). This process, commonly described as metabolic endotoxaemia, activates inflammatory pathways including toll-like receptor 4 (TLR4), nuclear factor-kappa B (NF-κB), and downstream cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).

Persistent inflammatory activation interferes with insulin receptor signalling through mechanisms including serine phosphorylation of insulin receptor substrate proteins, ultimately impairing insulin sensitivity in hepatic, adipose, and skeletal muscle tissues. In addition, microbiome-associated inflammation contributes to adipose tissue macrophage activation and dysregulated adipokine secretion, further amplifying metabolic dysfunction (18, 19).

However, the relationship between inflammation, insulin resistance, and the microbiome is likely bidirectional, as obesity, hyperglycaemia, and metabolic disease themselves can alter gut permeability and microbial ecology. Consequently, distinguishing causal microbial effects from secondary metabolic alterations remains a major challenge in human studies (19).

3.3. Bile acid pathways in glucose regulation

Microbiome-mediated bile acid metabolism represents another important mechanism linking intestinal microbial activity with glucose regulation and metabolic homeostasis. Following hepatic synthesis, primary bile acids undergo microbial deconjugation and transformation into secondary bile acids within the intestine, generating metabolites capable of acting as endocrine signalling molecules (20) (figure 2). These bile acids interact predominantly with the farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5), both of which play significant roles in glucose and lipid metabolism.

FXR activation regulates hepatic gluconeogenesis, insulin sensitivity, and lipid homeostasis, whereas TGR5 signalling has been associated with enhanced glucagon-like peptide-1 secretion, increased energy expenditure, and improved insulin responsiveness (20).

Through modulation of these receptor-mediated pathways, gut microbial alterations may influence the development and progression of obesity, type 2 diabetes mellitus, and metabolic dysfunction-associated steatotic liver disease (figure 2).

Nevertheless, considerable interindividual variability in bile acid composition, influenced by diet, medication exposure, genetics, and obesity status, complicates interpretation of human data and limits definitive conclusions regarding the precise contribution of microbiome-derived bile acid signalling to metabolic disease pathogenesis (20–21).

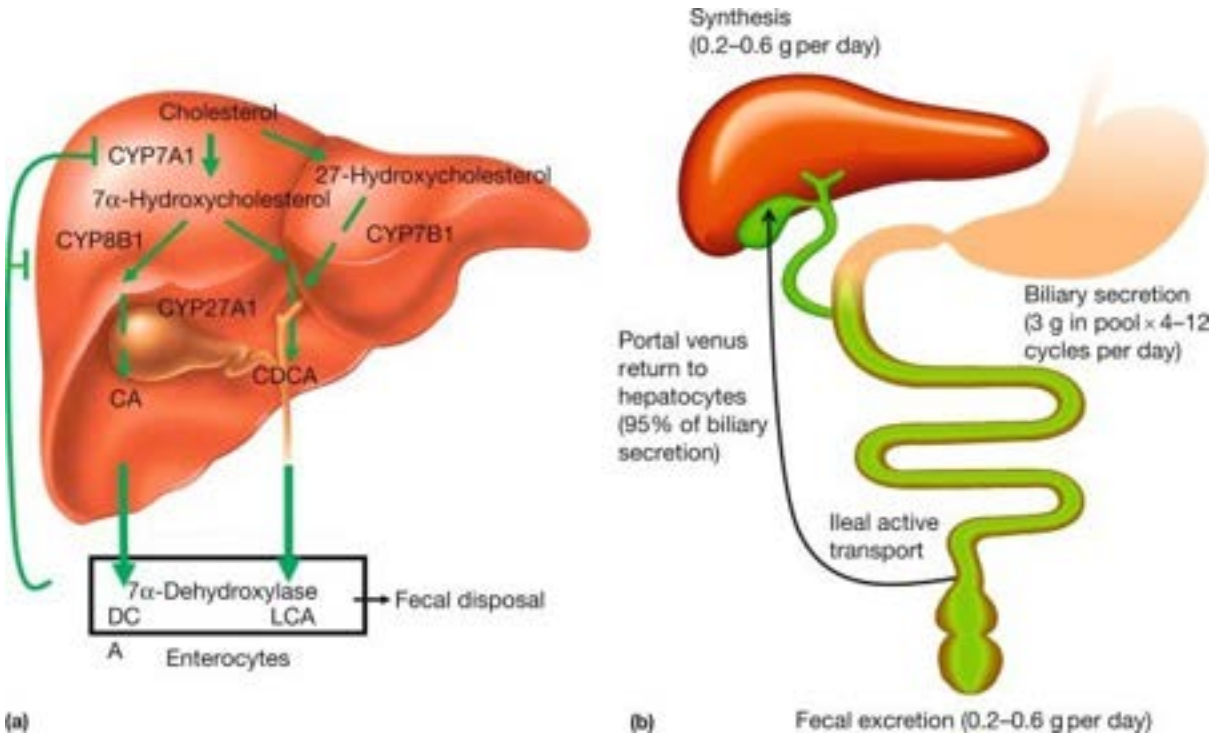

Figure 2. Bile acid metabolism

3.4. Human observational and interventional evidence — causality debate

Human observational studies have identified associations between insulin resistance and alterations in gut microbial diversity, reduced abundance of short-chain fatty acid-producing bacteria, and shifts in taxa such as Akkermansia muciniphila and Firmicutes/Bacteroidetes populations. In parallel, interventional approaches including dietary modification, probiotics, prebiotics, and faecal microbiota transplantation (FMT) have demonstrated varying degrees of metabolic improvement, including enhanced insulin sensitivity and altered glucose homeostasis in selected populations (22).

However, findings across studies remain inconsistent, and the extent to which microbial alterations directly contribute to metabolic dysfunction remains unresolved. Establishing causality is complicated by substantial confounding factors including obesity, dietary patterns, medication exposure, particularly metformin use, physical activity, and host genetic variability, all of which independently influence microbiome composition and metabolic outcomes (23).

Furthermore, methodological heterogeneity in microbiome sequencing techniques, bioinformatic analyses, and endocrine phenotyping continues to limit reproducibility across studies. While current evidence strongly supports mechanistic plausibility for microbiome involvement in insulin resistance, translation into clinically actionable and causally established therapeutic strategies remains an ongoing challenge.

4. Microbiome regulation of HPA axis activity and cortisol signalling

4.1. Experimental evidence for microbiome–HPA axis communication

Experimental evidence strongly supports communication between the gut microbiome and the hypothalamic–pituitary–adrenal (HPA) axis. Germ-free animal studies have shown exaggerated corticosterone and

adrenocorticotropic hormone responses to stress, with partial normalisation after microbial colonisation during early life, suggesting that microbial exposure may influence HPA-axis development and stress responsivity (24).

More recent experimental work also indicates that microbial depletion can disturb stress-related signalling in brain regions such as the hippocampus and amygdala and alter HPA-axis rhythmicity, supporting the concept that the microbiome contributes not only to stress magnitude but also to circadian organisation of stress responses. However, these findings are largely derived from animal models, and their direct applicability to human cortisol physiology remains uncertain (25).

4.2. Microbial influence on glucocorticoid synthesis and metabolism

Beyond regulating central stress pathways, gut microbes may also influence glucocorticoid metabolism directly. Selected intestinal bacteria are capable of metabolising endogenous corticosteroids, including cortisol and corticosterone, through enzymatic transformations that may alter steroid bioavailability and downstream receptor signalling (26).

For example, microbial conversion of cortisol into 11β-hydroxyandrostenedione and related metabolites has been described, while microbial steroid metabolites may also interact with host enzymes such as 11β-hydroxysteroid dehydrogenase, which regulates local active glucocorticoid exposure (26, 27).

These findings suggest that the gut microbiome may participate in peripheral glucocorticoid metabolism, although the clinical relevance of these pathways in humans remains incompletely established.

4.3. Stress exposure, microbial diversity, and host susceptibility as modifiers

The relationship between stress, cortisol signalling, and the gut microbiome is highly contextdependent. Psychological and physiological stress can alter gut microbial composition, intestinal permeability, immune signalling, and gut–brain communication, while microbial profiles may in turn influence stress responsiveness (28).

Human studies suggest associations between cortisol levels and microbial diversity, but these relationships are modified by factors such as diet, early-life exposures, medication use, circadian rhythm, host genetics, and baseline psychological vulnerability. Therefore, microbial effects on the HPA axis are unlikely to be uniform across individuals; instead, they may depend on host susceptibility and environmental context (28, 29). This variability may partly explain why psychobiotic and probiotic trials show strain-specific and inconsistent effects on stressrelated outcomes and cortisol measures.

4.4. Discordance between animal models and clinical associations

Although animal studies provide strong mechanistic evidence that the microbiome can regulate HPA-axis activity, human clinical evidence remains less consistent. Rodent models allow controlled manipulation of microbial exposure, stress paradigms, and host genetics, but human cortisol regulation is shaped by complex behavioural, social, dietary, circadian, and psychological factors that are difficult to isolate (30).

In addition, many human studies are cross-sectional, use heterogeneous microbiome profiling methods, and measure cortisol at limited time points despite its strong diurnal variation. As a result, observed

associations between microbial diversity, stress exposure, and cortisol signalling cannot reliably establish causality.

The current evidence therefore supports a biologically plausible microbiome–HPA axis connection, but its clinical significance in humans requires stronger longitudinal, mechanistic, and intervention-based studies (30).

5. The estrobolome and oestrogen metabolism

5.1. Estrobolome definition and β-glucuronidase-mediated oestrogen deconjugation

The estrobolome refers to the collection of gut microbial genes and enzymes involved in oestrogen metabolism, particularly those capable of modifying conjugated oestrogens excreted into the intestine. A central mechanism involves bacterial β-glucuronidase enzymes, which deconjugate oestrogen glucuronides and convert them back into biologically active forms that may be reabsorbed into circulation. This process suggests that gut microbial enzymatic activity can influence systemic oestrogen bioavailability and downstream oestrogen-sensitive physiology (31, 32) (Figure 3).

However, β-glucuronidase activity is not specific to oestrogens alone, and metagenomic detection of β-glucuronidase genes does not necessarily confirm functional oestrogen deconjugation, making careful interpretation essential.

5.2. Enterohepatic recycling of oestrogens and systemic hormonal implications

Oestrogens undergo hepatic conjugation through glucuronidation and sulfation, which increases water solubility and enables biliary excretion into the gut. Once in the intestine, microbial enzymes can deconjugate these metabolites, allowing unconjugated oestrogens to be reabsorbed through enterohepatic circulation (33).

Through this pathway, the gut microbiome may contribute to variation in circulating oestrogen levels, particularly in settings where ovarian production is reduced, such as menopause. These mechanisms have potential implications for bone health, reproductive function, and oestrogensensitive tissues, but current evidence remains largely associative and is influenced by host age, menopausal status, adiposity, diet, and medication exposure (33, 34).

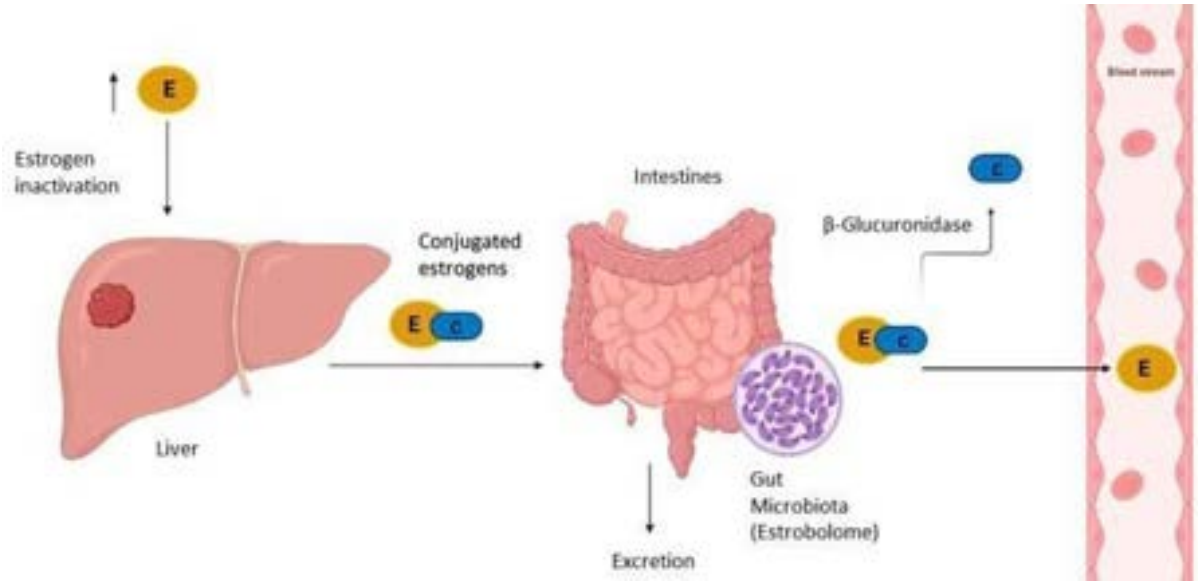

Figure 3. Estrogen metabolism involving gut microbiota (estrobolome).

5.3. Links to reproductive disorders (PCOS, endometriosis, oestrogen-dependent cancers)

Alterations in gut microbiome composition and estrobolome activity have been proposed as contributors to reproductive and hormone-sensitive disorders, including polycystic ovary syndrome, endometriosis, and oestrogen-dependent cancers. In endometriosis, gut dysbiosis and altered microbial oestrogen metabolism may interact with inflammation and immune dysfunction, although causal pathways remain unclear (35).

In hormone-dependent cancers, particularly breast cancer, microbial regulation of oestrogen recycling has been investigated as a possible modifier of systemic oestrogen exposure and tumour-relevant hormonal signalling.

However, these associations should not be interpreted deterministically, as reproductive and oncological disorders are multifactorial and shaped by genetics, adiposity, immune regulation, exogenous hormones, and environmental exposures (35, 36).

5.4. Population-level inconsistencies and confounders

Despite strong mechanistic plausibility, population-level studies of the estrobolome remain inconsistent. Differences in sequencing methods, enzyme assays, faecal sampling, metabolomic approaches, and definitions of “estrobolome activity” limit comparability across studies. Important confounders include age, menopausal status, menstrual cycle phase, body composition, diet, antibiotic exposure, oral contraceptive use, hormone therapy, and metabolic disease (37).

In addition, measuring faecal β-glucuronidase activity may not accurately reflect tissuespecific oestrogen exposure or systemic endocrine outcomes. Therefore, while the estrobolome provides a useful framework for understanding host–microbe interactions in oestrogen metabolism, stronger longitudinal and intervention-based studies are needed before clinical conclusions can be made.

6. Microbiome regulation of appetite and energy balance

6.1. Enteroendocrine hormone signalling (GLP-1, PYY, ghrelin)

The gut microbiome may influence appetite and energy balance through modulation of enteroendocrine hormone secretion. Microbial metabolites, particularly short-chain fatty acids and bile acid derivatives, can stimulate enteroendocrine cells to release glucagon-like peptide1 and peptide YY, hormones involved in satiety, insulin secretion, delayed gastric emptying, and postprandial glucose regulation.

Conversely, ghrelin, an orexigenic hormone primarily produced in the stomach, may also be influenced indirectly by microbial composition, nutritional status, and gut-derived signalling. These pathways suggest that microbial activity can shape appetite regulation through endocrine mechanisms, although human responses vary substantially depending on diet, baseline microbiome composition, obesity status, and metabolic health (38, 39).

6.2. Neural pathways: vagal afferents and the gut–brain axis

In addition to hormonal signalling, the microbiome communicates with appetite-regulating brain circuits through neural pathways, particularly vagal afferents. Enteroendocrine cells act as sensory intermediaries by detecting luminal nutrients and microbial metabolites and transmitting signals to the central nervous system through both endocrine and neural routes. This gut–brain

communication can influence satiety, reward-related feeding behaviour, gastrointestinal motility, and stress-linked eating patterns (40).

Experimental studies support microbiome-dependent modulation of vagal and enteroendocrine signalling, but translation to human appetite regulation remains complex because eating behaviour is shaped by psychological, social, metabolic, and environmental factors (41).

6.3. Microbial metabolite influence on satiety and food intake behaviour

Microbial metabolites provide a mechanistic link between gut microbial ecology and host feeding behaviour. Short-chain fatty acids may promote satiety through GLP-1 and PYY secretion, improve gut barrier integrity, and influence hypothalamic appetite pathways (42).

Bile acid metabolites can also regulate energy balance through TGR5-mediated incretin signalling, while microbial products derived from amino acid and tryptophan metabolism may affect gut–brain communication and behavioural responses to food.

However, these mechanisms are not uniformly beneficial or harmful; their effects depend on metabolite concentration, site of production, host receptor expression, and baseline metabolic state. As a result, microbial regulation of appetite should be viewed as context-dependent rather than as a simple "microbiome controls hunger" model (42, 43).

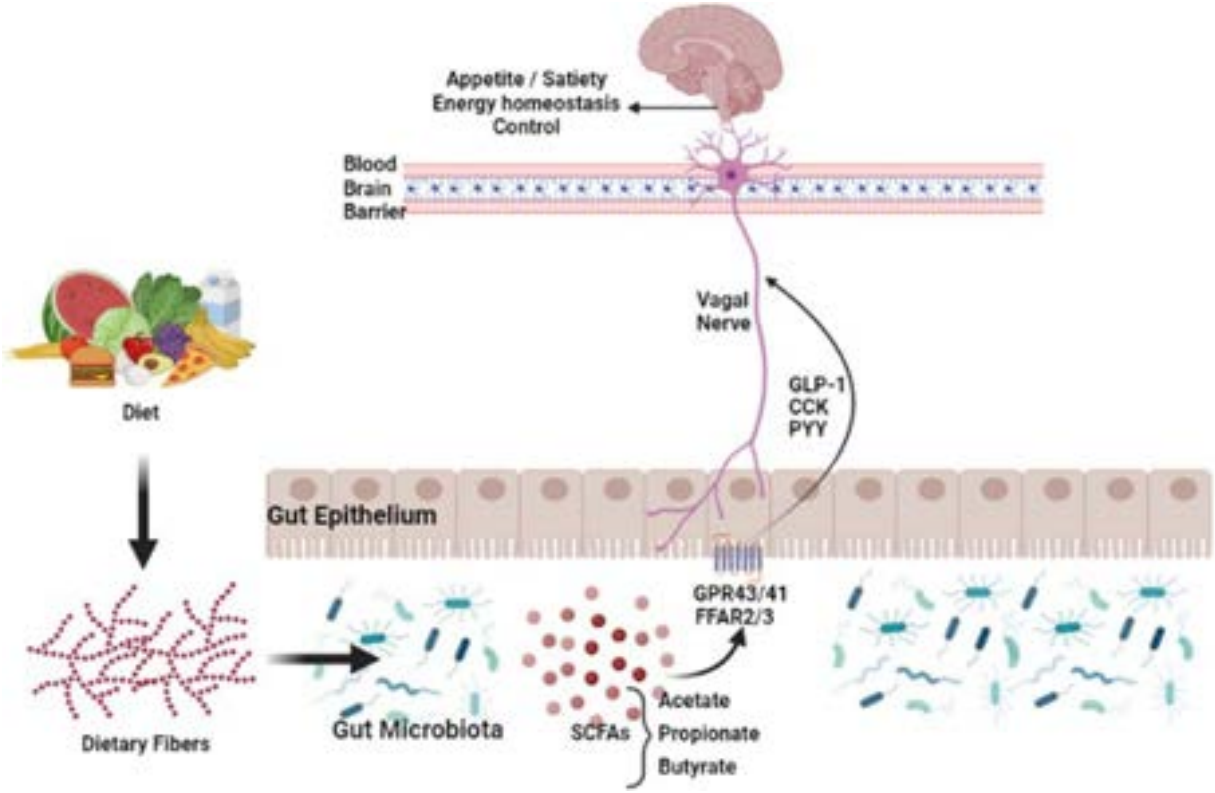

Figure 4. Dietary Fiber–Gut Microbiota–Brain Axis in the Regulation of Appetite and Energy Homeostasis

6.4. Interindividual variability in dietary and probiotic modulation

Dietary and probiotic interventions may alter appetite-related microbial metabolites and enteroendocrine signalling, but responses are highly individualised. Baseline microbiome composition, habitual diet, fibre intake, genetics, medication exposure, metabolic status, and adherence all influence whether an intervention produces meaningful changes in appetite, body weight, or glucose regulation (44, 45).

Probiotic effects are also strain-specific, meaning findings from one organism or formulation cannot be generalised across all probiotics. This variability may partly explain why clinical trials of microbiome-directed appetite interventions often show modest or inconsistent effects. Future approaches may require personalised dietary or microbiome-based strategies rather than universal supplementation models (45).

7. Cross-domain integration: shared pathways and bidirectionality

7.1. Overlapping microbial taxa and metabolites across endocrine domains

Across endocrine domains, several microbial metabolites appear repeatedly as shared mechanistic mediators, including short-chain fatty acids, bile acid derivatives, inflammatory microbial products, and tryptophan-derived metabolites. These signals may influence insulin sensitivity, appetite regulation, stress responsiveness, and sex hormone metabolism through overlapping pathways involving enteroendocrine cells, immune activation, epithelial barrier function, and neuroendocrine signalling (46).

Although specific taxa such as Akkermansia, Bifidobacterium, Lactobacillus, and SCFA producing bacteria are frequently discussed in endocrine contexts, taxonomic findings are often inconsistent across populations. Therefore, functional microbial activity may be more informative than taxonomy alone when evaluating endocrine effects (47).

7.2. Bidirectionality – how endocrine dysfunction reshapes the microbiome

The gut–endocrine axis is bidirectional: microbial signals may influence endocrine physiology, but endocrine dysfunction can also reshape the microbiome. Hyperglycaemia, obesity, chronic stress, altered cortisol rhythms, sex hormone changes, and inflammation may modify gut permeability, bile acid pools, immune tone, and microbial ecological niches (48).

For example, metabolic disease can alter gut barrier function and microbial composition, while menopausal oestrogen decline has been associated with changes in microbial diversity and oestrogen-metabolising capacity. This bidirectionality complicates causal interpretation, because microbiome alterations observed in endocrine disorders may represent drivers, consequences, or adaptive responses to disease (49).

7.3. Sex, age, and life-course as modulators of the gut–endocrine axis

Sex, age, and life-course stage are important modifiers of gut–endocrine interactions. Puberty, pregnancy, menopause, ageing, adiposity changes, and medication exposure can all alter both hormonal profiles and gut microbial composition. Sex steroids may influence microbial ecology, while microbial enzymes may modify oestrogen recycling, creating reciprocal interactions that vary across reproductive stages (50).

Ageing is also associated with changes in microbial diversity, immune function, metabolic regulation, and endocrine resilience, which may modify susceptibility to insulin resistance, appetite dysregulation, stress-related endocrine changes, and hormone-sensitive disease.

These life-course effects highlight the need for endocrine microbiome studies to stratify by sex, age, menopausal status, and metabolic phenotype rather than treating the microbiome as a uniform exposure across populations (51).

8. Methodological challenges and translational gaps

8.1. Heterogeneity in microbiome profiling methodologies

A major limitation in microbiome–endocrine research is the substantial heterogeneity in microbiome profiling methodologies, which complicates reproducibility and cross-study comparison. Studies differ in sample collection procedures, DNA extraction protocols, sequencing platforms, taxonomic databases, bioinformatic pipelines, and statistical approaches, all of which can influence reported microbial composition and diversity (52).

Furthermore, many earlier studies relied predominantly on 16S ribosomal RNA sequencing, which provides limited functional resolution compared with shotgun metagenomics, metabolomics, and transcriptomic approaches. Differences in dietary assessment, medication reporting, and timing of sample acquisition further contribute to variability in endocrine-related findings (52). Consequently, inconsistent methodological approaches remain a major barrier to identifying reproducible microbial signatures associated with endocrine physiology and disease.

8.2. Limitations of animal-to-human translation

Animal models have provided important mechanistic insights into microbiome–endocrine interactions, particularly regarding insulin signalling, HPA-axis regulation, and gut–brain communication. However, translation of these findings into human physiology remains challenging. Germ-free and antibiotic-treated rodent models allow controlled experimental manipulation but do not fully replicate the complexity of human microbial ecology, dietary diversity, environmental exposures, or behavioural influences (53).

In addition, rodent endocrine physiology, circadian rhythms, bile acid composition, and immune responses differ substantially from those of humans, limiting direct extrapolation of mechanistic findings. Many experimental studies also use highly controlled diets and homogeneous genetic backgrounds that fail to reflect real-world human variability (53, 54). As a result, mechanisms that appear robust in preclinical models may produce inconsistent or attenuated effects in clinical settings.

8.3. Observational confounding and reverse causality

Most human evidence linking the gut microbiome to endocrine outcomes is observational, making causal interpretation inherently difficult. Microbial composition is influenced by numerous confounding factors including diet, obesity, medications, age, physical activity, smoking, socioeconomic status, stress exposure, and host genetics, many of which independently affect endocrine physiology (55). For example, metformin, proton pump inhibitors, antibiotics, and hormonal therapies can significantly alter gut microbial profiles, complicating interpretation of disease-associated microbial signatures.

Reverse causality also remains a major concern, as endocrine disorders themselves may modify gut permeability, immune signalling, gastrointestinal motility, and microbial ecology. Consequently, microbiome alterations observed in metabolic or hormonal

disease states may represent contributors, consequences, or adaptive responses rather than primary causal drivers (56). These challenges highlight the need for carefully controlled longitudinal and mechanistic human studies.

8.4. Lack of standardised endocrine phenotyping in microbiome studies

Another important limitation is the lack of standardised endocrine phenotyping across microbiome studies. Many investigations rely on single biomarkers or broad clinical diagnoses without adequately characterising hormonal rhythms, metabolic status, reproductive stage, or endocrine heterogeneity within patient populations (57).

For instance, cortisol exhibits marked circadian variation, insulin resistance can be assessed using multiple non-equivalent indices, and reproductive hormone profiles vary across menstrual and menopausal stages. Inconsistent definitions of metabolic syndrome, obesity phenotypes, and endocrine disease severity further limit comparability between studies.

Moreover, microbiome analyses are frequently performed without integrating detailed hormonal, inflammatory, metabolomic, or clinical phenotyping, reducing the ability to establish biologically meaningful host–microbe relationships. Standardised multidimensional endocrine assessment will therefore be essential for improving interpretability and translational relevance in future microbiome research (58).

9. Future directions

9.1. Longitudinal and life-course study designs

Future microbiome–endocrine research should prioritise longitudinal and life-course study designs capable of capturing temporal changes in both microbial composition and endocrine physiology. Cross-sectional analyses provide limited insight into causality and cannot adequately distinguish whether microbial alterations precede, accompany, or result from endocrine dysfunction (59).

Longitudinal approaches following individuals across developmental and hormonal transitions, including puberty, pregnancy, menopause, ageing, and metabolic disease progression, may provide a more comprehensive understanding of dynamic host–microbe interactions. Such designs may also help identify critical windows during which microbial modulation exerts greater endocrine effects or therapeutic responsiveness.

9.2. Integration of microbiome profiling with endocrine phenotyping

Advancing the field will require integration of microbiome profiling with detailed endocrine, metabolic, inflammatory, and clinical phenotyping. Combining metagenomics with metabolomics, transcriptomics, hormonal assays, and immune profiling may provide greater functional insight than taxonomic analysis alone (60).

In particular, characterising microbial metabolites, receptor signalling pathways, and host endocrine responses simultaneously may improve identification of biologically relevant host–microbe interactions. Standardised phenotyping frameworks incorporating circadian hormone assessment, metabolic indices, reproductive staging, dietary evaluation, and medication exposure will also be essential for improving reproducibility and clinical interpretation across studies (60, 61).

9.3. Interventional trials: probiotics, prebiotics, dietary modulation

Interventional strategies targeting the gut microbiome, including probiotics, prebiotics, dietary modification, synbiotics, and

faecal microbiota transplantation, continue to attract interest as potential approaches for modulating endocrine and metabolic health. Experimental and early clinical studies suggest that selected interventions may influence insulin sensitivity, appetite regulation, inflammatory signalling, and stress-related endocrine pathways (62).

However, clinical outcomes remain inconsistent due to strain-specific effects, heterogeneity in study design, small sample sizes, and marked interindividual variability in microbial responsiveness. Future trials should therefore prioritise standardised intervention protocols, adequately powered cohorts, longer follow-up durations, and mechanistically informed outcome measures integrating both microbial and endocrine endpoints (62–64).

9.4. Precision medicine applications

The growing recognition of interindividual variability in microbiome composition and endocrine responses has generated interest in precision medicine approaches targeting the gut– endocrine axis. Personalised dietary strategies, microbiome-guided therapeutics, metabolitebased interventions, and targeted microbial modulation may eventually allow more individualised management of metabolic and endocrine disorders (65).

Advances in artificial intelligence, systems biology, and multi-omics integration may further improve prediction of therapeutic responsiveness and identification of clinically relevant microbial signatures (65). Nevertheless, significant translational barriers remain, including methodological standardisation, validation across diverse populations, regulatory considerations, and the need to establish causal rather than associative microbial mechanisms before routine clinical implementation can be justified.

10. Conclusion

The gut microbiome has emerged as an important regulator of endocrine physiology through complex interactions involving microbial metabolites, immune signalling pathways, enteroendocrine communication, and neuroendocrine crosstalk. Accumulating evidence suggests that microbial-derived short-chain fatty acids, bile acid metabolites, inflammatory mediators, and estrobolome-associated enzymes may influence insulin sensitivity, glucose homeostasis, hypothalamic–pituitary–adrenal axis activity, oestrogen metabolism, appetite regulation, and energy balance.

However, despite strong mechanistic plausibility and substantial experimental support, translation into consistent and clinically actionable human evidence remains limited by methodological heterogeneity, observational confounding, interindividual variability, and unresolved issues of causality and bidirectionality.

Importantly, the gut–endocrine axis should not be viewed as a unidirectional pathway in which microbial alterations simply drive endocrine disease. Rather, endocrine dysfunction itself can reshape microbial ecology through changes in metabolism, inflammation, hormonal signalling, gut permeability, and host physiology, creating dynamic and context-dependent host–microbe interactions.

This bidirectional complexity likely contributes to the inconsistent microbial signatures observed across endocrine disorders and highlights the limitations of reductionist or taxonomyfocused approaches.

Future progress in the field will depend on longitudinal and mechanistically integrated research combining microbiome profiling with detailed endocrine phenotyping, metabolomics, immune characterisation,

and clinical assessment. Carefully designed interventional trials and systemslevel analytical approaches will be necessary to distinguish causal microbial mechanisms from secondary disease-associated changes.

Although significant translational challenges remain, continued advances in multiomics technologies and precision medicine may ultimately support development of personalised microbiome-targeted strategies for metabolic, reproductive, and neuroendocrine disorders.

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- **Financial Relationships:** The authors have no financial relationships with any organisations, companies, pharmaceutical industries, or institutions that could have influenced the content of this manuscript.
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- **Data Availability:** No primary datasets were generated or analysed during the preparation of this narrative review. All information discussed was derived from previously published literature.
- **Ethical Approval:** Ethical approval was not required because this study is a narrative review based exclusively on previously published studies and publicly available data.
- **Human and Animal Subjects:** This manuscript did not involve human participants, patient data, animal subjects, or experimental interventions.
- **Informed Consent:** Informed consent was not required as no identifiable patient information or participant data were included.
- **Author Contributions:** All authors contributed to the conceptualisation, literature review, drafting, revision, and final approval of the manuscript.
- **Generative AI Statement:** No generative artificial intelligence tools were used to generate scientific content, interpret findings, or formulate conclusions in this manuscript. AI-assisted tools, if used, were limited to minor language or grammatical refinement only.
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Transient osteoporosis of the hip during pregnancy – a case report

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Transient osteoporosis of the hip is a rare condition that commonly affects pregnant women during the third trimester. The presentation includes acute onset of hip pain and functional limitation. It may be misdiagnosed because of non-specific clinical findings. Magnetic resonance imaging plays a crucial role in establishing an early and accurate diagnosis. The aim of this case report is to describe the clinical presentation, imaging findings and postpartum follow-up of a 32-year-old pregnant woman diagnosed with transient osteoporosis of the hip. The patient presented with progressive unilateral hip pain during late pregnancy, with no history of trauma. Clinical examination revealed restriction of movement due to pain. Magnetic resonance imaging of the pelvis and hip joints was performed to investigate the cause. Follow-up imaging was obtained to assess disease evolution after delivery. Magnetic resonance imaging demonstrated diffuse bone marrow edema involving the femoral head and neck. Joint congruency was preserved with no evidence of osteonecrosis or fracture, consistent with osteoporosis of the hip. Postpartum follow-up revealed clinical improvement confirming the transient nature of the condition. Hence, transient osteoporosis of the hip should be considered in pregnant women with non-traumatic hip pain. Magnetic resonance imaging is the diagnostic modality of choice and follow-up confirms spontaneous resolution.
Keywords: *Osteoporosis, Bone marrow edema, postpartum recovery.*

Introduction

Transient osteoporosis of the hip is a rare, self-limiting condition characterised by acute or progressive onset of hip pain, functional limitation, and reversible bone marrow edema on magnetic resonance imaging (MRI). It most commonly affects middle-aged men and women in the third trimester of pregnancy. The condition usually resolves spontaneously however, it is clinically important because it may be misdiagnosed due to its nonspecific presentation and overlap with other causes of hip pain, particularly avascular necrosis. (1) Early recognition is crucial as the prognosis and management of transient osteoporosis differ significantly from those of avascular necrosis and other serious differentials such as stress fracture and septic arthritis. (2) In pregnancy, diagnosis may be further delayed because imaging options are more limited, and symptoms may be attributed to normal musculoskeletal changes of gestation.

This case highlights the importance of MRI in establishing the diagnosis and in differentiating transient osteoporosis of the hip from more progressive pathologies.

Case presentation

A 32-year-old pregnant woman with no known previous musculoskeletal disease presented with progressive unilateral hip pain during late pregnancy. The pain had an insidious onset, was localised in the hip region, and progressively worsened with movement and weight-bearing. Over time, it caused increasing limitation in mobility and daily activities. There was no history of trauma or injury. The patient reported no fever or other systemic symptoms, no prior similar episodes and no known inflammatory or infectious disease.

Initial evaluation included plain radiography of the affected hip. The X-ray did not demonstrate significant abnormalities. There was no evidence of fracture, no evident joint space narrowing and no clear radiographic signs of osteopenia. However, due to persistent symptoms and the known limitations of plain radiography in early bone marrow

pathology, further imaging was pursued with MRI.

MRI of the hips demonstrated hyperintense signal in the head and neck region of the right femur on T2-STIR coronal and axial images, consistent with diffuse bone marrow edema. Mild right-sided joint effusion was also present (Figure 1). These findings supported the diagnosis of transient osteoporosis of the hip. Importantly, there was no evidence of focal subchondral collapse or destructive changes to suggest advanced avascular necrosis.

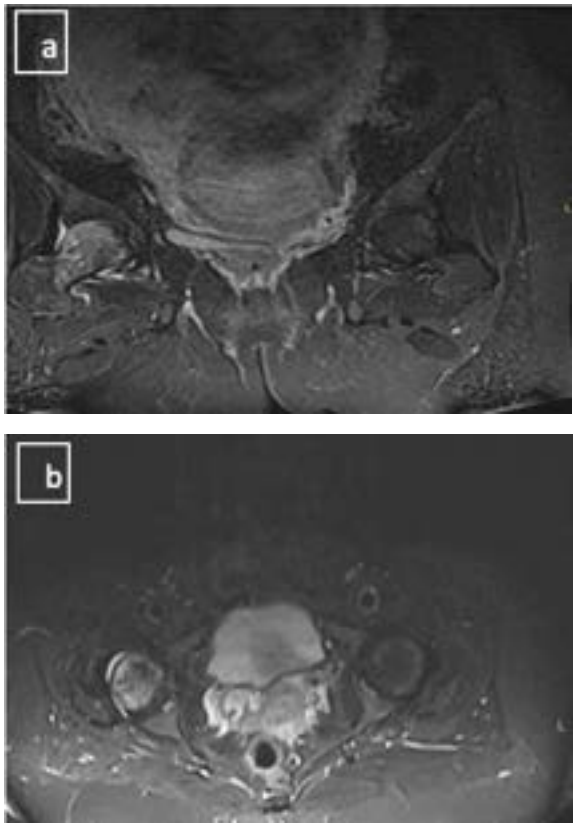

Figure 1a. and 1b. T2-stir-coronal and axial images-show hyperintense signals in the head and neck region of the right femur due to marrow edema, mild joint effusion on the right side.

Based on the clinical and imaging findings, conservative management was undertaken. The patient was advised activity modification and reduced weight-bearing, with analgesia for pain control and close clinical follow-up during pregnancy. Follow-up MRI showed normalisation of signal in the head and neck region of the right femur on T2-STIR coronal and axial images (Figure 2).

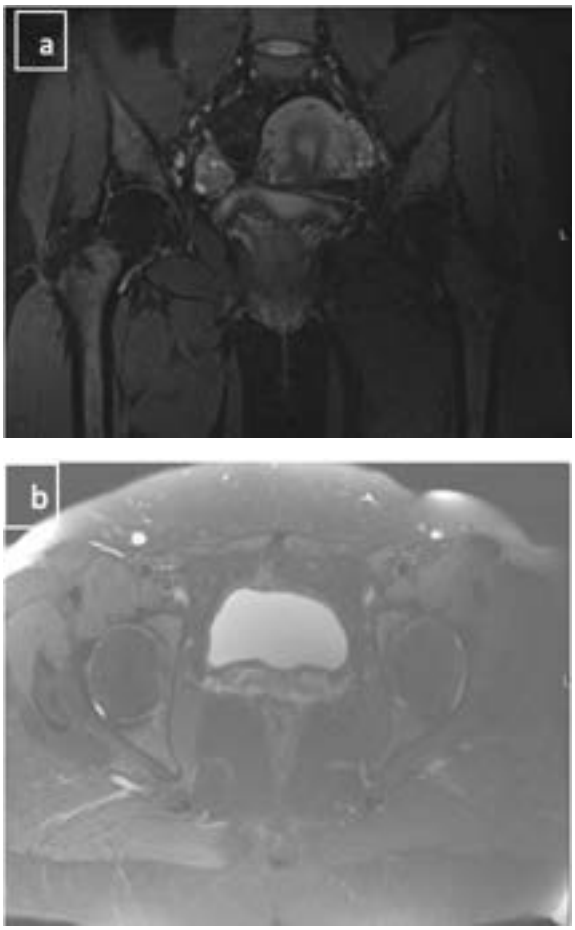

Figure 2a. and 2b. T2-stir-coronal and axial images – show normal signals in the head and neck region of the right femur after delivery

Discussion

Transient osteoporosis of the hip is a rare cause of atraumatic hip pain during pregnancy. Its pathophysiology remains incompletely understood, however, vascular, hormonal, and mechanical factors have all been proposed. Some studies reveal that genetic factors also play a role. (3) The condition is better understood radiologically, with MRI demonstrating the hallmark reversible marrow signal abnormalities. Patients usually describe unilateral groin or hip pain of gradual onset which is worsened by weight-bearing. In a Japanese study, it was reported that 20% of cases presented with bilateral hip

involvement. (4) Plain radiographs are commonly normal early in the disease, as occurred in this patient. MRI is a key diagnostic modality because it is highly sensitive of bone marrow edema and its ability to detect abnormalities before radiographic osteopenia becomes visible. (5)

The major diagnostic challenge is distinguishing transient osteoporosis of the hip from avascular necrosis. This distinction is vital because avascular necrosis may progress to subchondral collapse and permanent joint destruction whereas transient osteoporosis of the hip is self-limiting. (2) MRI is crucial to this differentiation. Other differential diagnosis includes stress fracture and septic arthritis however, in these cases, edema alone will not be presented as there would be other clinical or imaging findings. (6) Management is generally conservative with reduced weight-bearing, activity modification, and analgesia forming the mainstay of treatment. There is limited data available regarding the preferred mode of delivery for women with transient osteoporosis of the hip but, one study reported that caesarean section can limit birth-related trauma and complications of transient osteoporosis of the hip. (7)

Conclusion

This case reinforces several important clinical points. Firstly, persistent atraumatic hip pain during pregnancy should not be dismissed as a routine discomfort of gestation. Secondly, a normal X-ray does not exclude important pathologies. Third, MRI is essential not only for diagnosis but also for distinguishing transient osteoporosis from avascular necrosis and thereby preventing misdiagnosis and unnecessary intervention. MRI allows early diagnosis which allows appropriate conservative management, avoids unnecessary intervention, and supports favourable outcomes.

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Endocrine disruptors in everyday life microplastics and PFAS as modulators of reproductive, thyroid, and metabolic health via the gut-endocrine axis

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Introduction: Microplastics and per- and polyfluoroalkyl substances (PFAS) are pervasive environmental contaminants increasingly recognised for their endocrine-disrupting potential. Chronic low-dose exposure has raised concerns regarding reproductive, thyroid, and metabolic dysfunction, while emerging evidence implicates the gut–endocrine axis as a key pathway linking environmental exposure to systemic hormonal and metabolic disturbances. **Aim:** To critically synthesise mechanistic, experimental, and epidemiological evidence linking microplastics and PFAS to endocrine dysfunction, with emphasis on reproductive, thyroid, and metabolic health and the role of the gut–endocrine axis. **Materials and methods:** A narrative review was conducted using evidence from epidemiological studies, human biomonitoring data, animal models, and in-vitro mechanistic research. Findings were evaluated across reproductive, thyroid, and metabolic domains and interpreted in the context of exposure variability, mixture effects, and methodological limitations. **Results and discussion:** Current evidence demonstrates biologically plausible endocrine-disrupting effects of microplastics and PFAS through mechanisms including nuclear receptor perturbation, oxidative stress, inflammatory signalling, altered steroidogenesis, and epigenetic modification. Reproductive effects include impaired gametogenesis, fertility disturbances, and adverse pregnancy outcomes. Thyroid associations are common but inconsistent, likely reflecting non-linear dose responses and exposure heterogeneity. Metabolic evidence supports obesogenic and diabetogenic potential through insulin resistance, adipogenesis, lipid dysregulation, and stress-axis activation. Emerging data further suggest that gut microbiota dysbiosis may amplify endocrine dysfunction via altered bile acid metabolism, intestinal permeability, and microbial hormone biotransformation. **Conclusion:** Current evidence supports endocrine-disrupting plausibility for microplastics and PFAS across reproductive, thyroid, and metabolic systems, with the gut–endocrine axis emerging as an important integrative pathway. However, causal inference remains limited by methodological heterogeneity and insufficient longitudinal human data. Future research should prioritise harmonised exposure assessment, mixture-aware life-course designs, and translational integration of mechanistic and clinical evidence.

Keywords: *microplastics; PFAS; endocrine disruption; gut microbiota; reproductive health; thyroid dysfunction; metabolic disease*

1. Introduction

1.1. Ubiquity of microplastics and PFAS in human environments and biological matrices

Microplastics and per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants now detected across virtually all ecological and human biological systems. Microplastics arise from degradation of larger plastic materials and are widely distributed in food, drinking water, and atmospheric particulate matter, while PFAS persist because of highly stable carbon–fluorine bonds and extensive industrial use (1, 2).

Human biomonitoring studies have identified both contaminants in blood, urine, placenta, breast milk, semen, and fetal tissues, supporting concerns regarding chronic systemic exposure and transplacental transfer. Importantly, exposure occurs through complex mixtures rather than isolated compounds, complicating exposure assessment and toxicological interpretation. Their environmental persistence, bioaccumulative potential, and continuous low-dose exposure patterns have therefore positioned microplastics and PFAS as major emerging threats to endocrine and metabolic health (3).

1.2. Endocrine-disrupting chemical (EDC) framework and relevance to chronic low-dose exposure

The endocrine-disrupting chemical (EDC) framework is particularly relevant to microplastics and PFAS because biologically significant effects may occur at environmentally relevant concentrations rather than overtly toxic doses. Both contaminants demonstrate the capacity to interfere with endocrine signalling through receptor perturbation, altered steroidogenesis, disruption of hormone transport, oxidative stress, inflammatory activation, and epigenetic modification (4).

Experimental evidence implicates interactions with oestrogen, androgen, thyroid hormone, and peroxisome proliferator-activated receptors, linking exposure to reproductive, thyroid, and metabolic dysfunction (5). Importantly, endocrine disruption frequently exhibits non-monotonic dose-response relationships and heightened vulnerability during developmental windows, limiting the applicability of traditional toxicological models.

Furthermore, chronic low-dose and mixed-compound exposures more accurately reflect real-world human conditions than acute single-agent paradigms, underscoring substantial limitations in current risk-assessment frameworks (2, 3–5).

1.3. Rationale for a life-course and systems-biology approach

The health effects of endocrine-disrupting contaminants are increasingly understood within a life-course framework in which exposure timing critically influences disease susceptibility. Fetal development, infancy, puberty, and pregnancy represent vulnerable periods characterised by tightly regulated endocrine signalling, rendering these stages particularly sensitive to environmental perturbation (6).

Emerging evidence further suggests that the biological consequences of microplastics and PFAS extend beyond isolated endocrine pathways and involve interconnected immune, metabolic, microbial, and inflammatory networks.

Systems-biology approaches have therefore become essential for understanding how chronic environmental exposures contribute to multifactorial disease states. Particular attention has focused on the gut–endocrine axis, as alterations in gut microbiota composition, intestinal permeability, bile acid metabolism, and microbial hormone biotransformation may amplify systemic endocrine dysfunction (7). This integrative framework provides a more biologically plausible model for understanding the long-term consequences of chronic environmental contaminant exposure.

1.4. Aim and scope of the review

This narrative review critically synthesises current mechanistic, experimental, and epidemiological evidence linking microplastics and PFAS to reproductive, thyroid, and metabolic dysfunction. Particular emphasis is placed on the gut–endocrine axis as an emerging integrative pathway mediating endocrine and metabolic disruption. Evidence from human biomonitoring studies, observational research, animal models, and in-vitro investigations is evaluated to assess mechanistic plausibility, translational relevance, and consistency across endocrine domains.

In addition, major methodological limitations including exposure misclassification, mixture complexity, and challenges in causal inference are critically examined to contextualise current evidence and identify priorities for future research.

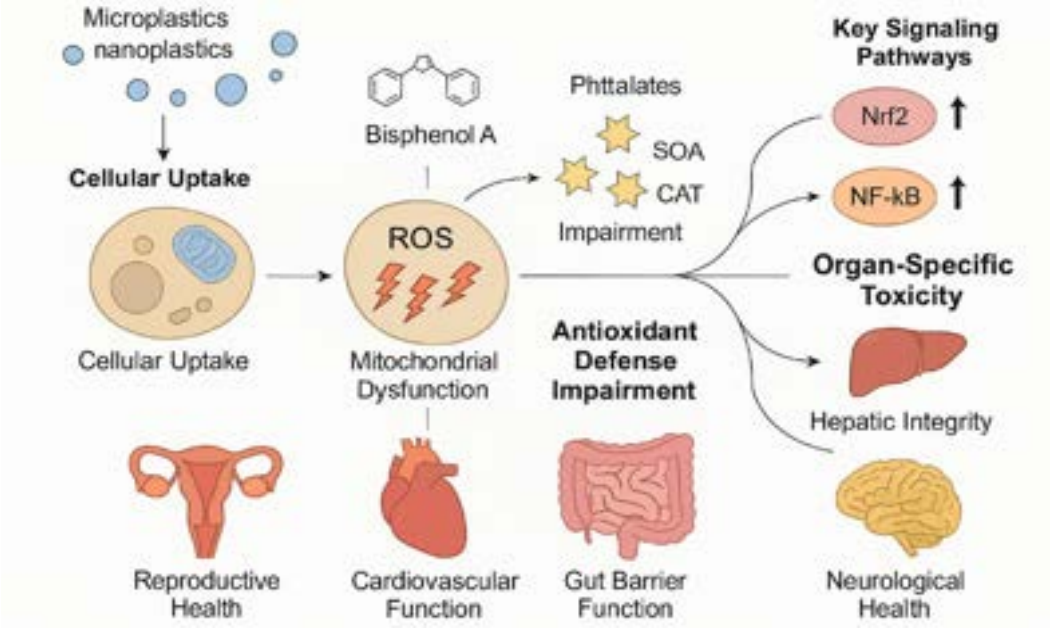

Figure 1. Microplastics, endocrine disruptors, and oxidative stress

2. Sources, exposure pathways, and human biomonitoring

2.1. Environmental distribution and persistence of microplastics and PFAS

Microplastics and PFAS exhibit exceptional environmental persistence, facilitating widespread distribution across aquatic, terrestrial, atmospheric, and biological systems. Microplastics originate from both primary industrial particles and secondary fragmentation of larger plastic materials, leading to extensive contamination of marine ecosystems, agricultural soils, freshwater sources, and indoor environments 8). PFAS persistence is driven by highly stable carbon–fluorine bonds that resist thermal, chemical, and biological degradation, resulting in prolonged environmental half-lives and global dissemination even in remote regions.

Both contaminants demonstrate substantial mobility within ecological systems and capacity for trophic transfer, raising concerns regarding cumulative bioaccumulation across food chains (9). Importantly, environmental persistence promotes continuous low-dose human exposure, while co-occurrence with other pollutants may further modify toxicity through additive or synergistic interactions inadequately captured by conventional environmental monitoring frameworks.

2.2. Routes of human exposure (dietary, inhalational, dermal, and occupational)

Human exposure to microplastics and PFAS occurs through multiple overlapping pathways, with dietary intake considered a dominant source for many populations. Contamination of seafood, drinking water, packaged foods, and processed products contributes substantially to oral exposure, while inhalation of airborne microplastic fibres and PFAS-containing particulate matter represents an increasingly recognised route, particularly within urban and indoor environments (10) (figure 2). Dermal exposure may occur through cosmetics, textiles, and consumer products, although its quantitative contribution appears lower than oral and inhalational pathways for most compounds.

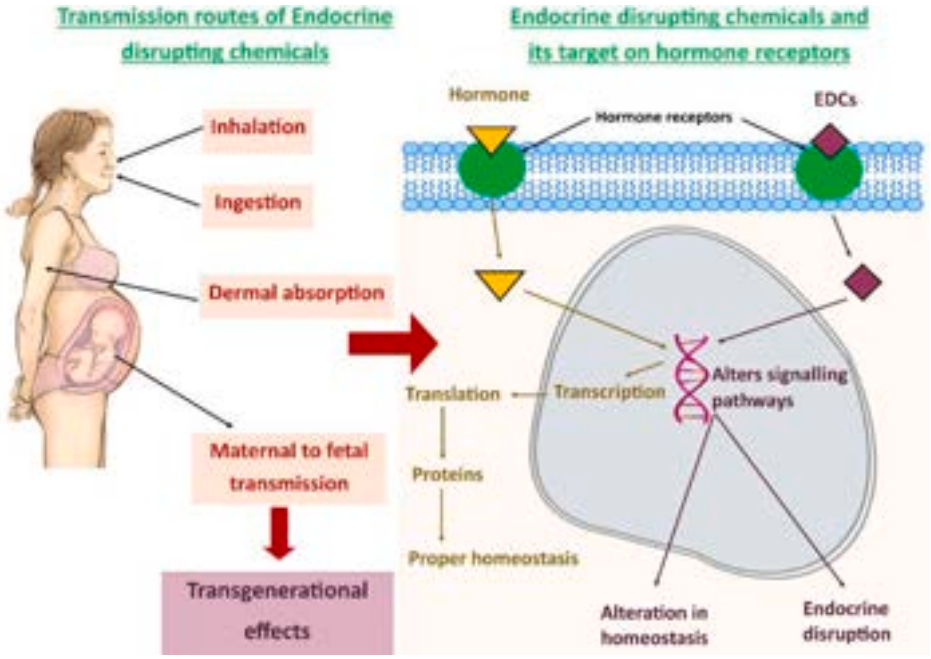

Figure 2. Routes of endocrine disrupters

Occupational exposure remains particularly relevant in industrial manufacturing, firefighting, waste management, and textile sectors, where contaminant concentrations may substantially exceed general population levels. Importantly, exposure patterns vary according to geography, socioeconomic conditions, dietary behaviours, and regulatory environments, complicating population-level risk estimation and reinforcing the need for mixture-aware exposure models reflecting real-world conditions (10–12).

2.3. Human biomonitoring data and exposure metrics

Human biomonitoring studies increasingly demonstrate widespread internal exposure to both microplastics and PFAS, with contaminants identified in blood, urine, breast milk, placental tissue, semen, and fetal compartments. PFAS biomonitoring is relatively advanced because of established analytical methods and longer biological half-lives, enabling more consistent exposure quantification across populations (13–14).

In contrast, microplastic biomonitoring remains methodologically heterogeneous due to variability in particle size definitions, sampling protocols, contamination control, and analytical detection techniques.

Current evidence nonetheless supports systemic absorption and translocation beyond the gastrointestinal tract, challenging earlier assumptions that microplastics primarily exert local intestinal effects. Importantly, conventional biomonitoring metrics may inadequately capture cumulative exposure burden, biologically active metabolites, or critical exposure windows, thereby limiting mechanistic interpretation and epidemiological comparability (15). These limitations are particularly relevant given increasing recognition that endocrine-disrupting effects may occur at low concentrations not reflected by traditional toxicological thresholds.

2.4. Challenges in exposure assessment and inter-study variability

Accurate exposure assessment remains a major limitation within environmental endocrine research involving microplastics and PFAS. Considerable heterogeneity exists across studies regarding sampling

methodologies, analytical platforms, exposure definitions, and outcome measures, substantially limiting cross-study comparability and meta-analytic synthesis. Microplastic research is particularly constrained by lack of standardised particle classification systems and high susceptibility to background contamination during sample collection and analysis (16).

PFAS investigations face additional complexity because of the large number of structurally diverse compounds, evolving replacement chemicals, and varying toxicokinetic properties. Furthermore, most epidemiological studies rely on single time-point measurements that may inadequately reflect chronic exposure trajectories or vulnerable developmental windows (17).

Confounding from co-exposures, socioeconomic factors, dietary behaviours, and lifestyle variables further complicates causal inference. Collectively, these methodological limitations contribute to inconsistent findings across endocrine outcomes and highlight the need for harmonised, longitudinal, and mixture-aware exposure assessment frameworks.

3. Mechanisms of endocrine disruption

3.1. Nuclear receptor perturbation (ER, AR, TR, and PPARγ)

Microplastics, PFAS, and associated chemical additives exert endocrine-disrupting effects partly through direct and indirect perturbation of nuclear receptor signalling pathways. Experimental evidence demonstrates interactions with oestrogen receptors (ER), androgen receptors (AR), thyroid hormone receptors (TR), and peroxisome proliferator-activated receptor gamma (PPARγ), thereby influencing reproductive, thyroid, and metabolic homeostasis (18). PFAS compounds in particular have shown affinity for PPAR signalling pathways involved in adipogenesis, lipid metabolism, and insulin sensitivity, supporting mechanistic links to obesogenic and diabetogenic phenotypes (figure 3).

Certain plastic-associated compounds additionally demonstrate oestrogenic or antiandrogenic activity capable of disrupting gonadal development and steroid-responsive tissues. Importantly, receptor interactions frequently occur at environmentally relevant concentrations and may exhibit non-monotonic dose-response relationships, challenging traditional toxicological assumptions regarding threshold-dependent toxicity (19).

The complexity of mixed-compound exposures further complicates mechanistic interpretation, as receptor crosstalk and additive endocrine effects may amplify biological disruption beyond that observed in single-agent models (18–20).

3.2. Disruption of hormone synthesis, transport, and signalling

Beyond receptor-level interactions, microplastics and PFAS can disrupt endocrine homeostasis through interference with hormone synthesis, transport, metabolism, and downstream signalling pathways. Experimental studies demonstrate altered expression and activity of steroidogenic enzymes involved in oestrogen, androgen, and cortisol biosynthesis, alongside disruption of thyroid hormone synthesis and peripheral conversion. PFAS have additionally been associated with altered binding to serum transport proteins, including transthyretin and albumin, potentially modifying hormone bioavailability and tissue distribution (21). These disruptions may impair endocrine feedback regulation and contribute to dysregulated hypothalamic–pituitary signalling across multiple hormonal axes.

Importantly, endocrine effects may arise not only from parent compounds but also from plastic-associated additives, degradation products, and contaminant mixtures, further complicating mechanistic attribution (22). The cumulative consequence is a multifaceted disturbance of endocrine signalling networks with potential implications for fertility, metabolic regulation, neurodevelopment, and chronic disease susceptibility.

3.3. Oxidative stress and inflammatory signalling pathways

Oxidative stress and chronic inflammatory activation are increasingly recognised as central mechanisms linking environmental contaminant exposure to endocrine and metabolic dysfunction. Both microplastics and PFAS have been shown to induce excessive reactive oxygen species generation, mitochondrial dysfunction, lipid peroxidation, and impaired antioxidant defence systems across experimental models (23) (figure 1).

These processes activate inflammatory signalling cascades involving nuclear factor kappa B (NF-κB), cytokine production, and inflammasome pathways, thereby promoting systemic low-grade inflammation associated with insulin resistance, reproductive dysfunction, and thyroid dysregulation. Microplastic-induced epithelial injury and PFAS-mediated immune modulation may further compromise intestinal barrier integrity, amplifying inflammatory signalling through increased microbial translocation and gut dysbiosis (24) (figure 1).

Importantly, oxidative and inflammatory mechanisms may act synergistically with endocrine receptor perturbation and epigenetic modification, suggesting that endocrine disruption represents a complex network phenomenon rather than an isolated receptor-mediated process.

3.4. Epigenetic programming and transgenerational effects

Emerging evidence suggests that endocrine-disrupting contaminants may exert long-term biological effects through epigenetic modification and developmental reprogramming mechanisms. Experimental studies have demonstrated that exposure to microplastics and PFAS can alter DNA methylation patterns, histone modification, chromatin structure, and non-coding RNA expression, thereby influencing gene regulation without changing underlying DNA sequences. Such alterations may persist beyond the initial exposure period and affect pathways involved in reproduction, metabolism, inflammation, and endocrine signaling (25).

Of particular concern is evidence that exposure during critical developmental windows including fetal life and early childhood may induce durable physiological changes with implications extending into adulthood and potentially subsequent generations. Although human evidence remains limited and causality difficult to establish, epigenetic mechanisms provide a biologically plausible framework linking chronic low-dose environmental exposure to long-term endocrine and metabolic disease susceptibility (26).

4. Reproductive health effects

4.1. Disrupted steroidogenesis and gonadal function

Experimental and epidemiological evidence increasingly implicates microplastics and PFAS in disruption of steroidogenesis and gonadal endocrine function. Both contaminant classes have been associated with altered expression and activity of key steroidogenic enzymes, including aromatase, 3β-hydroxysteroid dehydrogenase, and cytochrome P450 family proteins, leading to

dysregulated synthesis of oestrogens, androgens, and progesterone.

PFAS-mediated activation of nuclear receptor pathways and microplastic-associated chemical additives may further impair hypothalamic–pituitary–gonadal axis signalling, thereby altering gonadotropin regulation and reproductive hormone homeostasis (27).

Experimental models demonstrate ovarian follicular dysfunction, reduced testosterone production, Leydig and Sertoli cell injury, and impaired gonadal development, although translational consistency in human studies remains variable because of exposure heterogeneity and confounding factors (28). Nonetheless, the convergence of mechanistic and biomonitoring evidence supports biologically plausible reproductive endocrine toxicity associated with chronic environmental exposure.

4.2. Impaired spermatogenesis and oogenesis

Microplastics and PFAS have been increasingly associated with impaired gametogenesis through mechanisms involving oxidative stress, mitochondrial dysfunction, endocrine disruption, and inflammatory signalling. Animal and in-vitro studies consistently demonstrate reduced sperm count, impaired motility, abnormal morphology, DNA fragmentation, and testicular structural damage following contaminant exposure. Similarly, ovarian toxicity has been linked to disrupted folliculogenesis, impaired oocyte maturation, granulosa cell apoptosis, and reduced ovarian reserve (29).

Emerging evidence also suggests that nanoplastic particles may cross biological barriers and directly accumulate within reproductive tissues, potentially amplifying local cellular toxicity. Human evidence remains less definitive but increasingly supports associations between PFAS exposure and altered semen quality, delayed reproductive maturation, and diminished ovarian function (30).

However, inconsistencies across observational studies reflect methodological limitations, including variability in exposure assessment, small sample sizes, and difficulty isolating the effects of mixed environmental contaminants.

4.3. Fertility and reproductive outcomes

Chronic exposure to endocrine-disrupting contaminants has been linked to adverse fertility and reproductive outcomes across both male and female populations. Epidemiological studies have associated higher PFAS concentrations with prolonged time-to-pregnancy, reduced fecundability, menstrual irregularities, and impaired assisted reproductive technology outcomes, although effect estimates remain heterogeneous.

Microplastic exposure has similarly been implicated in reproductive dysfunction through inflammatory and endocrine-mediated mechanisms, though direct human evidence remains comparatively limited (31). Importantly, reproductive toxicity likely reflects cumulative disruption across multiple biological pathways, including hormonal imbalance, oxidative stress, gamete impairment, and altered endometrial receptivity.

Evidence further suggests that combined contaminant exposures may produce additive or synergistic reproductive effects inadequately captured by single-compound analyses. While causal inference remains constrained by observational study design and exposure complexity, current data increasingly support reproductive vulnerability to chronic low-dose environmental contaminant exposure (32).

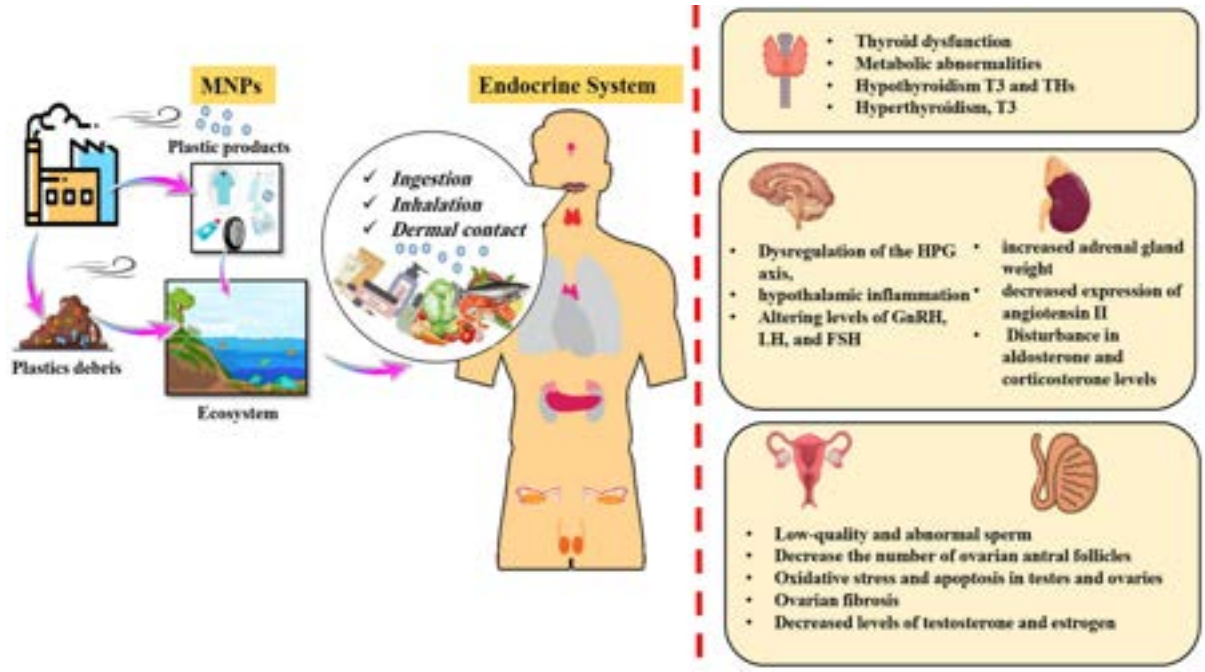

Figure 3. Summary of profound impact of microplastics on endocrine health

4.4. Pregnancy-related and developmental effects

Pregnancy represents a highly sensitive physiological state during which endocrine disruption may affect both maternal health and fetal development. PFAS and microplastics have been detected in placental tissue, amniotic fluid, cord blood, and fetal compartments, demonstrating the capacity for transplacental transfer during critical developmental windows. Observational studies have linked PFAS exposure to adverse outcomes including gestational hypertension, altered fetal growth, preterm birth, pregnancy loss, and impaired neurodevelopment, although findings remain inconsistent across populations (33).

Experimental evidence further suggests that contaminant-induced oxidative stress, placental inflammation, vascular dysfunction, and endocrine perturbation may impair placental function and fetal programming. Early-life exposure is of particular concern because developmental endocrine disruption may induce long-term metabolic, reproductive, and neuroendocrine consequences extending into adulthood (33–34).

Despite increasing concern, substantial uncertainty remains regarding dose thresholds, mixture interactions, and long-term developmental implications in humans.

4.5. Compound-mixture effects and exposure-window sensitivity

Real-world environmental exposure occurs through complex contaminant mixtures rather than isolated compounds, yet most toxicological research continues to evaluate single-agent effects. This reductionist approach may underestimate biological risk, as PFAS, microplastics, plastic additives, and co-existing environmental pollutants may interact through additive, synergistic, or antagonistic mechanisms (35).

Exposure timing further critically influences reproductive toxicity, with fetal development, puberty, and pregnancy representing particularly vulnerable windows characterised by tightly regulated endocrine signalling and developmental plasticity. Experimental studies demonstrate that low-dose exposures during these sensitive periods

may induce disproportionate and potentially irreversible physiological effects relative to adult exposure (35, 36). Moreover, sex-specific susceptibility, genetic variability, nutritional status, and co-existing environmental stressors may further modify reproductive outcomes.

Collectively, these observations reinforce the need for life-course and mixture-aware frameworks capable of more accurately reflecting real-world endocrine-disrupting exposure patterns.

5. Thyroid health effects

5.1. Mechanisms of thyroid-axis disruption

The thyroid axis appears particularly vulnerable to environmental endocrine disruption because thyroid hormone homeostasis depends on tightly regulated synthesis, transport, metabolism, and receptor signalling pathways. PFAS and certain plastic-associated compounds can interfere with thyroid physiology through multiple mechanisms, including receptor antagonism, altered iodine utilisation, disruption of deiodinase activity, and competition with thyroid hormone transport proteins (37).

Structural similarity between some PFAS compounds and endogenous fatty acids may facilitate interactions with thyroid-related signalling pathways and carrier proteins such as transthyretin.

Experimental studies additionally demonstrate oxidative stress, mitochondrial dysfunction, and inflammatory activation within thyroid tissues following exposure. Importantly, thyroid disruption may have systemic consequences extending beyond endocrine regulation because thyroid hormones critically influence neurodevelopment, metabolism, cardiovascular function, and reproductive health (38).

5.2. Effects on thyroid hormone synthesis and transport

Experimental evidence suggests that microplastics and PFAS may disrupt thyroid hormone synthesis, metabolism, and systemic transport through several interconnected pathways. PFAS exposure has been associated with altered circulating concentrations of thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH), although findings remain inconsistent across studies (39). Mechanistically, PFAS may impair iodine uptake, interfere with thyroid peroxidase activity, and competitively bind serum transport proteins, thereby altering hormone bioavailability and tissue distribution.

Certain microplastic-associated additives may additionally disrupt thyroid receptor signalling and peripheral hormone metabolism. Importantly, even subtle alterations in thyroid hormone homeostasis may carry significant physiological implications, particularly during fetal and early-life development when thyroid hormones play essential roles in neurodevelopmental programming. However, variability in exposure metrics, population characteristics, and analytical methods continues to complicate interpretation of observed associations (40).

5.3. Epidemiological associations and inconsistencies

Epidemiological studies examining associations between PFAS, microplastics, and thyroid dysfunction have produced mixed but increasingly concerning findings. Several cohort and cross-sectional investigations report associations between higher PFAS exposure and altered TSH or thyroid hormone concentrations, increased prevalence of hypothyroidism, and disrupted thyroid homeostasis during pregnancy and childhood. However, effect direction and

magnitude vary considerably across populations, with some studies demonstrating inverse associations or null findings (41). This inconsistency likely reflects substantial heterogeneity in exposure assessment, demographic characteristics, nutritional iodine status, co-exposures, and endocrine susceptibility.

Evidence regarding microplastics remains comparatively limited and largely experimental, with human epidemiological data still emerging. Importantly, many studies rely on single time-point biomonitoring measurements that may inadequately capture chronic exposure trajectories or sensitive developmental windows, limiting causal interpretation (42).

5.4. Non-linear dose-response relationships and susceptibility modifiers

Thyroid-disrupting effects of environmental contaminants frequently exhibit non-monotonic dose-response relationships in which low-dose exposures may produce disproportionate biological effects not predicted by traditional toxicological models. Such patterns are particularly relevant for endocrine-active compounds because hormonal systems operate through highly sensitive feedback mechanisms vulnerable to subtle perturbation (43). Individual susceptibility is further modified by developmental stage, sex, pregnancy status, iodine sufficiency, genetic polymorphisms, nutritional factors, and co-existing environmental exposures.

Fetal and pediatric populations appear especially vulnerable because thyroid hormones are essential for neurodevelopment and metabolic regulation during early life. These complexities complicate epidemiological interpretation and challenge conventional regulatory frameworks based primarily on threshold-dependent toxicity assumptions (44).

Consequently, understanding thyroid disruption associated with microplastics and PFAS requires integrative models accounting for exposure timing, biological susceptibility, and mixed-contaminant interactions.

6. Metabolic health effects

6.1. Obesogenic mechanisms and adipogenesis

Microplastics and PFAS are increasingly recognised as potential obesogens capable of promoting adipose tissue expansion and metabolic dysfunction through endocrine-mediated mechanisms. Experimental studies demonstrate activation of peroxisome proliferator-activated receptor gamma (PPARγ) signalling pathways, enhanced adipocyte differentiation, altered lipid storage, and dysregulated energy homeostasis following PFAS exposure. Microplastic-associated additives may similarly influence adipogenesis through inflammatory and oxidative pathways (45).

Importantly, obesogenic effects may occur at environmentally relevant concentrations and during critical developmental periods, supporting the developmental origins of metabolic disease hypothesis. Chronic low-dose exposure may therefore contribute to long-term metabolic programming rather than acute toxicity alone. However, translating experimental findings into human risk estimates remains challenging because obesity is multifactorial and influenced by substantial behavioural, dietary, and socioeconomic confounding (46).

6.2. Insulin resistance and glucose dysregulation

Accumulating evidence links environmental endocrine disruptors to impaired glucose homeostasis and insulin resistance.

PFAS exposure has been associated with altered insulin signalling, pancreatic β-cell dysfunction, impaired glucose tolerance, and increased risk of type 2 diabetes in several observational studies, although findings remain heterogeneous across populations. Proposed mechanisms include oxidative stress, mitochondrial dysfunction, inflammatory activation, and disruption of insulin-responsive signalling pathways (47).

Experimental studies additionally suggest that gut microbiota alterations induced by microplastics and PFAS may contribute to systemic metabolic dysregulation through chronic low-grade inflammation and altered metabolite production. Importantly, metabolic effects may emerge gradually through cumulative exposure burden rather than immediate toxicity, complicating epidemiological detection and causal interpretation (48).

6.3. Lipid dysregulation and metabolic syndrome

PFAS exposure has been consistently associated with altered lipid metabolism, including elevated total cholesterol, low-density lipoprotein cholesterol, and triglyceride concentrations across multiple epidemiological studies.

Mechanistically, these effects are linked to disruption of hepatic lipid metabolism, altered fatty acid oxidation, PPAR-mediated signalling, and inflammatory activation. Microplastic exposure may similarly impair lipid homeostasis through oxidative and mitochondrial pathways, although human evidence remains limited (49).

Such disturbances may contribute to broader metabolic syndrome phenotypes characterised by dyslipidaemia, insulin resistance, central obesity, and cardiovascular risk. However, substantial inter-study variability persists because of differences in PFAS subclasses, exposure duration, population characteristics, and adjustment for lifestyle-related confounders. Nonetheless, current evidence increasingly supports a biologically plausible relationship between chronic environmental contaminant exposure and adverse cardiometabolic outcomes (50).

6.4. HPA-axis activation and stress–metabolic crosstalk

Emerging evidence suggests that endocrine-disrupting contaminants may influence metabolic homeostasis through dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and chronic stress signalling pathways. Experimental models demonstrate altered cortisol regulation, inflammatory activation, and neuroendocrine stress responses following PFAS and microplastic exposure. Persistent HPA-axis activation may promote insulin resistance, visceral adiposity, appetite dysregulation, and systemic inflammation, thereby contributing to metabolic disease progression (51).

Interactions between stress signalling, immune activation, and gut microbiota disruption further highlight the interconnected nature of endocrine and metabolic regulation. Importantly, psychosocial stressors, socioeconomic factors, and co-existing environmental exposures may amplify susceptibility to contaminant-induced metabolic dysfunction, reinforcing the need for integrative systems-level approaches in environmental endocrine research (51, 52).

7. The gut–endocrine axis as an integrative pathway

7.1. Microplastic- and PFAS-induced gut microbiota dysbiosis

Emerging evidence suggests that disruption of gut microbial ecology represents a central mechanism through which microplastics and PFAS may exert systemic

endocrine and metabolic effects. Experimental studies consistently demonstrate alterations in microbial diversity, taxonomic composition, and metabolic function following contaminant exposure, including reductions in beneficial commensal bacteria and expansion of pro-inflammatory microbial populations (53). These dysbiotic shifts have been associated with impaired short-chain fatty acid production, altered bile acid metabolism, oxidative stress, and intestinal inflammation.

PFAS exposure has additionally been linked to perturbations in microbial pathways involved in lipid and glucose metabolism, while microplastic particles may directly interact with intestinal surfaces and microbial communities, amplifying local inflammatory responses (54). Importantly, gut dysbiosis may serve not merely as a secondary consequence of exposure but as an active mediator of endocrine disruption through modulation of host metabolic, immune, and hormonal signalling networks.

7.2. Microbial regulation of bile acid and hormone metabolism

The gut microbiota plays a critical role in regulating bile acid homeostasis and hormone biotransformation, providing a mechanistic link between environmental exposures and systemic endocrine dysfunction. Microbial enzymes influence enterohepatic circulation, steroid hormone deconjugation, and bile acid signalling pathways that regulate lipid metabolism, glucose homeostasis, and inflammatory activity (55).

Disruption of microbial composition by PFAS and microplastics may therefore alter bile acid receptor activation, including farnesoid X receptor and Takeda G protein-coupled receptor 5 signalling, with downstream effects on metabolic and endocrine regulation.

Furthermore, microbial modulation of oestrogen metabolism through the estrobolome may influence reproductive and thyroid physiology, particularly under conditions of chronic dysbiosis. These interactions highlight the gut microbiota as a biologically plausible intermediary between environmental contaminant exposure and multisystem endocrine disruption (56).

7.3. Intestinal barrier dysfunction and systemic inflammatory signalling

Microplastics and PFAS may compromise intestinal barrier integrity through oxidative stress, epithelial injury, tight junction disruption, and inflammatory activation, thereby facilitating systemic translocation of microbial products and pro-inflammatory mediators. Experimental evidence demonstrates increased intestinal permeability and altered mucosal immune responses following contaminant exposure, promoting chronic low-grade inflammation implicated in metabolic, reproductive, and endocrine disease pathogenesis (57). Microplastic particles may directly damage epithelial surfaces, while PFAS-associated immune modulation can further impair intestinal homeostasis and mucosal defence mechanisms.

Increased permeability may additionally enhance systemic exposure to co-existing environmental toxicants and endotoxins, amplifying inflammatory signalling beyond the gastrointestinal tract. Importantly, intestinal barrier dysfunction provides a mechanistic bridge linking local gut dysbiosis to systemic endocrine and metabolic disturbances characteristic of chronic environmental contaminant exposure (58).

7.4. Gut–reproductive, gut–thyroid, and gut–metabolic crosstalk

The gut–endocrine axis functions through complex bidirectional interactions linking

microbial activity with reproductive, thyroid, and metabolic regulation. Gut microbiota composition influences sex hormone metabolism, hypothalamic–pituitary signalling, thyroid hormone availability, insulin sensitivity, and adipose tissue inflammation through integrated immune, neural, and metabolic pathways (59). Dysbiosis induced by microplastics and PFAS may therefore contribute simultaneously to reproductive dysfunction, thyroid dysregulation, and metabolic disease progression.

Emerging evidence suggests that microbial alterations affecting oestrogen recycling, bile acid signalling, inflammatory cytokine production, and microbial metabolite generation may collectively amplify systemic endocrine disruption. Importantly, these interconnected pathways challenge traditional organ-specific models of endocrine toxicity and support a systems-biology framework in which environmental exposures influence multiple endocrine domains through shared gut-mediated mechanisms (60).

8. Methodological challenges and limitations

8.1. Exposure misclassification and analytical heterogeneity

Exposure assessment remains a major limitation in research examining endocrine effects of microplastics and PFAS. Considerable heterogeneity exists across studies regarding sampling strategies, analytical methodologies, contaminant classification systems, and biomonitoring techniques, substantially limiting cross-study comparability. Microplastic research is particularly constrained by variability in particle size definitions, contamination control procedures, and detection thresholds, while PFAS investigations are complicated by the large number of structurally diverse compounds and evolving replacement chemicals (61).

Furthermore, many epidemiological studies rely on single time-point measurements that inadequately reflect cumulative exposure burden or sensitive developmental windows. Such methodological inconsistencies contribute significantly to exposure misclassification and may partially explain conflicting findings across endocrine outcomes (61, 62).

8.2. Challenges in assessing mixed-chemical and real-world exposures

Most environmental health studies continue to evaluate single compounds despite the reality that human exposure occurs through complex contaminant mixtures involving PFAS subclasses, microplastics, plastic additives, heavy metals, and other environmental toxicants (63). This reductionist approach may underestimate biological risk because mixed exposures can produce additive, synergistic, or antagonistic interactions across endocrine and inflammatory pathways.

Moreover, exposure profiles vary according to geography, diet, occupation, socioeconomic conditions, and regulatory environments, complicating generalisability across populations (63, 64). Existing toxicological frameworks therefore inadequately capture the dynamic and cumulative nature of real-world environmental exposures, highlighting the need for mixture-oriented models capable of integrating multiple simultaneous contaminant interactions.

8.3. Translational limitations between experimental and human evidence

Although experimental models provide important mechanistic insights, substantial translational gaps remain between laboratory findings and human clinical relevance.

Animal and in-vitro studies often involve exposure concentrations, particle characteristics, and exposure durations that may not accurately reflect human environmental conditions. Species-specific differences in metabolism, endocrine physiology, immune responses, and microbiome composition further limit extrapolation to human populations (65).

Additionally, controlled experimental settings cannot fully account for behavioural, dietary, genetic, and socioeconomic variables influencing human susceptibility. Consequently, mechanistic plausibility demonstrated experimentally does not necessarily establish causality in clinical populations, underscoring the need for integrative translational research combining mechanistic and longitudinal human evidence (65–67).

8.4. Confounding, reverse causality, and epidemiological constraints

Epidemiological investigations examining endocrine effects of microplastics and PFAS face substantial challenges related to confounding, reverse causality, and study design limitations. Lifestyle factors, dietary patterns, obesity, smoking, socioeconomic status, and co-existing environmental exposures may independently influence endocrine outcomes and exposure profiles, complicating causal interpretation.

Cross-sectional study designs predominate within the literature, limiting temporal inference and increasing vulnerability to reverse causality bias.

Additionally, many studies lack adequate adjustment for developmental timing, cumulative exposure burden, or endocrine susceptibility modifiers such as sex and pregnancy status. These methodological constraints contribute to inconsistent findings and emphasise the need for large-scale longitudinal cohorts incorporating repeated exposure assessment and mechanistically informed outcome measures (68, 69).

8.5. Lack of standardised endocrine and clinical outcome measures

Interpretation of current evidence is further limited by substantial heterogeneity in endocrine and clinical outcome assessment. Studies variably assess hormonal biomarkers, metabolic parameters, reproductive outcomes, thyroid indices, and inflammatory markers using inconsistent definitions and laboratory methodologies. Such variability complicates direct comparison across populations and weakens meta-analytic synthesis (70).

Furthermore, many endocrine-disrupting effects may manifest subclinically or through long-term physiological alterations not captured by conventional clinical endpoints. Limited integration of mechanistic biomarkers, microbiome profiling, and systems-level endocrine measures further constrains understanding of biological pathways linking exposure to disease. Establishing harmonised and clinically meaningful outcome frameworks therefore remains essential for advancing environmental endocrine research (71, 72).

9. Future directions

9.1. Harmonised frameworks for exposure assessment and biomonitoring

Future research requires standardised and harmonised frameworks for environmental exposure assessment to improve comparability across studies and strengthen causal inference. Development of validated protocols for microplastic sampling, particle classification, contamination control, and biomonitoring is particularly urgent given current analytical heterogeneity (73, 74).

For PFAS, broader assessment of

emerging replacement compounds and cumulative exposure burden beyond legacy chemicals is needed. Integrating repeated longitudinal measurements, internal dose biomarkers, and biologically relevant exposure metrics may further improve understanding of chronic low-dose exposure trajectories and vulnerable developmental windows (73–75).

9.2. Life-course and mixture-oriented study designs

Advancing environmental endocrine research will require life-course and mixture-aware study designs that more accurately reflect real-world exposure conditions. Prospective longitudinal cohorts incorporating prenatal, childhood, and adult exposure assessment are essential for identifying sensitive developmental windows and long-term disease trajectories (76).

Simultaneously, statistical and toxicological models capable of evaluating combined contaminant effects are needed to address the complexity of mixed environmental exposures. Such approaches may better capture cumulative endocrine burden and interactions between chemical, social, nutritional, and behavioural determinants of health (77).

9.3. Integration of multi-omics, microbiome profiling, and mechanistic biomarkers

Integration of multi-omics technologies offers substantial potential for advancing mechanistic understanding of endocrine disruption associated with microplastics and PFAS. Combining genomics, epigenomics, metabolomics, proteomics, and microbiome profiling may facilitate identification of early biological perturbations, exposure signatures, and disease susceptibility pathways (78).

Mechanistically informed biomarkers reflecting oxidative stress, inflammatory signalling, microbial metabolism, and endocrine pathway disruption may additionally improve translational interpretation of epidemiological findings. Such integrative approaches are particularly relevant for investigating the gut–endocrine axis as a systems-level mediator of environmental toxicity (78, 79).

9.4. Translational research linking mechanistic and clinical evidence

Bridging the gap between experimental toxicology and clinical epidemiology remains a major research priority. Future studies should integrate mechanistic laboratory findings with longitudinal human cohorts, clinical biomarkers, and systems-biology modelling to improve translational relevance. Incorporating realistic exposure conditions, environmentally relevant doses, and diverse population groups may strengthen external validity and inform risk assessment frameworks .

Greater interdisciplinary collaboration between toxicologists, endocrinologists, microbiome researchers, epidemiologists, and public health scientists will also be essential for developing clinically meaningful models of environmental endocrine disruption.

9.5. Regulatory, preventive, and public health implications

The widespread distribution and persistence of microplastics and PFAS raise important regulatory and public health concerns extending beyond traditional toxicological paradigms. Current evidence increasingly supports the need for precautionary approaches addressing chronic low-dose and mixed-contaminant exposures, particularly among vulnerable populations such as pregnant individuals and children.

Strengthening environmental regulation, reducing industrial emissions, improving food and water safety monitoring, and promoting safer material alternatives may help reduce cumulative exposure burden. Concurrently, public health strategies integrating environmental, metabolic, and endocrine health perspectives will be necessary to address the long-term implications of pervasive contaminant exposure within modern human environments.

10. Conclusion

Microplastics and per- and polyfluoroalkyl substances (PFAS) have emerged as pervasive environmental contaminants with biologically plausible endocrine-disrupting effects across reproductive, thyroid, and metabolic systems. Current mechanistic, experimental, and epidemiological evidence collectively suggests that chronic low-dose exposure may alter endocrine homeostasis through interconnected pathways involving nuclear receptor perturbation, oxidative stress, inflammatory signalling, epigenetic modification, and gut microbiota dysbiosis.

Increasing recognition of the gut–endocrine axis further supports a systems-biology model in which environmental exposures influence multiple physiological domains through integrated metabolic, microbial, immune, and hormonal networks.

Despite growing evidence, major uncertainties remain regarding causality, dose-response relationships, mixture interactions, and long-term clinical implications, largely because of methodological heterogeneity, exposure misclassification, and limitations within current epidemiological frameworks. Existing toxicological models may inadequately capture the complexity of chronic real-world exposures occurring across vulnerable developmental windows and within mixed-contaminant environments.

Consequently, future research must prioritise harmonised exposure assessment, longitudinal life-course designs, mechanistically informed biomarkers, and translational integration of experimental and human evidence. Advancing understanding of environmental endocrine disruption will be critical not only for refining regulatory policy and risk assessment but also for addressing the broader public health consequences of persistent contaminant exposure in contemporary human environments.

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Age-related alterations in eNOS signaling in distinct skeletal muscle types: protective effects of Aronia melanocarpa

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Introduction: Skeletal-muscle aging is progressive mitochondrial dysfunction, impaired-perfusion, redox imbalance, and chronic inflammation all contributing to sarcopenia and decline. Endothelial nitric-oxide synthase (eNOS) maintains vascular-homeostasis, nitric-oxide (NO) bioavailability, satellite-cell activation in skeletal-muscle, and more. Age-related reduction in eNOS activity decreases NO production, endothelial dysfunction, and impaired muscle regeneration. Evidence shows polyphenol foods regulate redox-sensitive pathways and improve eNOS-dependent signaling. Aronia-melanocarpa juice, rich in anthocyanins and proanthocyanidins, have vasoprotective and myoprotective effects. **Aim:** Evaluate the effect of a beverage from Aronia melanocarpa fruits, eNOS expression in three types of skeletal-muscle fast glycolytic (EDL), slow oxidative (SOL), and mixed (GAS) in aged rats. **Materials and methods:** Male Wistar rats (n = 24), divided into three groups: CY (3-month-old controls); CO (24-month-old controls); A (24-month-old rats supplemented with Aronia-melanocarpa juice, dose:10 ml/kg for 3 months). After experimental period, EDL, SOL, and GAS muscles dissected and immunohistochemically analyzed eNOS-experssion. **Results:** Age-related differences in eNOS expression were observed-in young and old controls across all muscle types. Aged-controls, reduced eNOS immunoreactivity detected in glycolytic fibers, particularly EDL and GAS muscles, indicating diminished NO signaling in fast-twitch fibers. Oxidative-fibers in SOL retained higher eNOS expression. Aronia-melanocarpa juice supplementation increased eNOS expression in peri- and epimysial regions and immunoreactivity in oxidative and intermediate fibers across all muscles. Gastrocnemius-muscle improved mostly, where oxidative-fibers restored eNOS levels compared to aged-controls. **Conclusion:** Polyphenol-rich Aronia-melanocarpa juice modulates eNOS skeletal-muscles in fiber-dependent manner. Restoration of eNOS immunoreactivity, oxidative-fibers, NO bioavailability and vascular support. Findings show myoprotective effect in polyphenol-rich supplementation and eNOS as molecular target mitigating skeletal-muscle aging.

Keywords: *aging, eNOS, NO, Skeletal muscle, oxidative stress*

Introduction

Aging of skeletal muscle is a complex biological process characterized by progressive mitochondrial dysfunction, impaired tissue perfusion, increased oxidative stress, and chronic low-grade inflammation, all of which contribute to the development of sarcopenia and decline in muscle function (1).

These alterations are closely associated with disruptions in vascular homeostasis and reduced efficiency of oxygen and nutrient delivery to muscle tissue. One of the central molecular regulators involved in these processes is endothelial nitric oxide synthase (eNOS), an enzyme responsible for the production of nitric oxide (NO), a key signaling molecule in skeletal muscle physiology and prone to decline with aging (2). The enzyme of eNOS is primarily expressed in the inner lining of endothelium blood vessels, where it is activated by shear stress, chemical triggers, and various biochemical stimuli creating NO from L-arginine and L-citrulline with important cofactors like tetrahydrobiopterin (BH4) (3, 4). From this process NO can help regulate the blood vessels by promoting vasodilation, exerting anti-atherosclerotic and anti-thrombotic effects (5, 6).

Increasing attention in recent years has been toward natural compounds with antioxidant properties, such as *Aronia melanocarpa*, a polyphenol-rich berry known for its gero protective potential (7, 8). *Aronia melanocarpa* fruits are considered promising natural anti-aging agents mainly because of their exceptionally high content of polyphenols, especially anthocyanins, flavonoids, and phenolic acids (9), (Fig. 1).

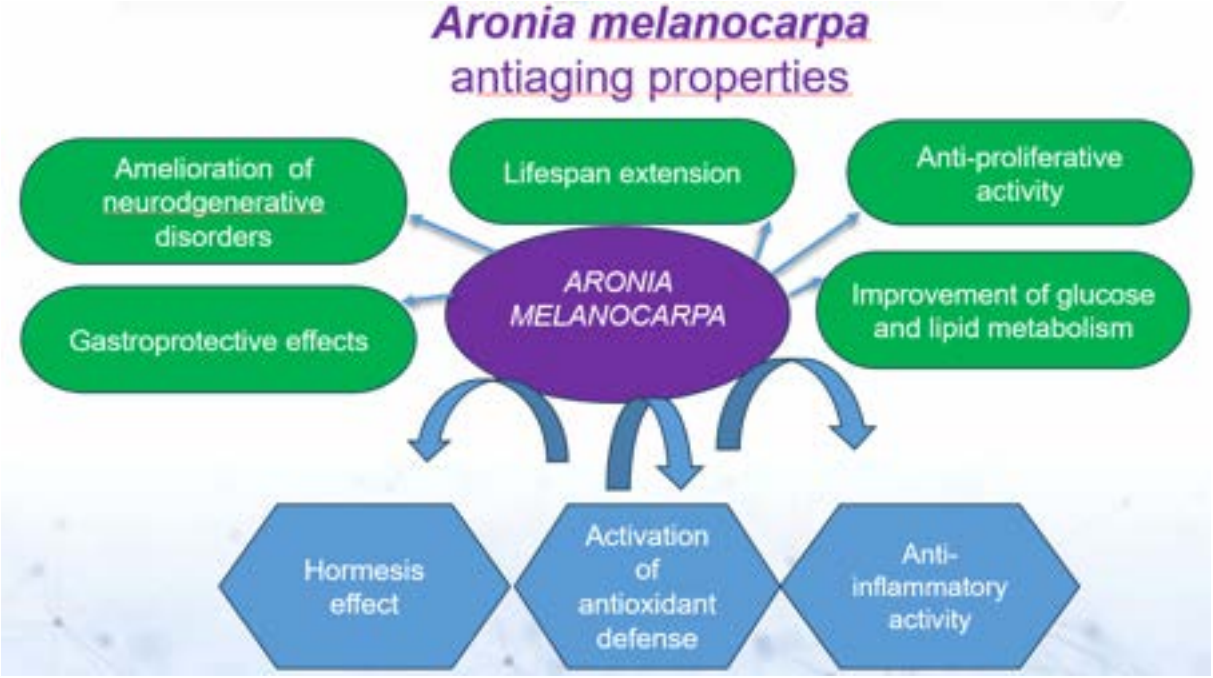

Figure 1. The anti-aging properties of *Aronia melanocarpa* and their mechanisms of action

Aim

The aim of present study was to evaluate the effect of a functional beverage based on *Aronia melanocarpa* fruits on eNOS expression in three types of skeletal muscle in naturally aged rats: fast glycolytic muscles (extensor digitorum longus muscle, EDL), slow oxidative (Soleus muscle, SOL), and mixed type muscles (Gastrocnemius muscle, GAS).

Materials and methods

The aronia fruit juice was produced from frozen berries in the Laboratory of Bioactive Compounds at the Institute of Organic Chemistry with a Center for Phytochemistry of the Bulgarian Academy of Sciences in Plovdiv, Bulgaria. Male Wistar rats (n = 24) were divided into three groups: CY (3-month-old controls); CO (27-month-old controls); and A (27-month-old rats orally supplemented with *Aronia melanocarpa* juice at a dose of 10 ml/kg for 3 months. After the experimental period, Extensor digitorum longus muscle (EDL), Soleus muscle (SOL), And Gastrocnemius muscle (GAS) were dissected and subjected to immunohistochemical analysis for eNOS expression. A semi-quantitative analysis was performed on the microphotographic images of the eNOS immunohistochemical reaction. Immunoreactivity was evaluated using a semi-quantitative scoring system (0–3) +/ – weak intensity; ++ – moderate intensity; +++ – strong intensity. The analysis was based on the intensity of the immune response and the percentage of the image area over which it was distributed.

Results

Table 1 and figure 2 present the results of the semi-quantitative analysis of the intensity of the eNOS immune response in the three experimental groups.

Table 1. Semi-quantitative analysis of eNOS immunoreactivity in skeletal muscle types (EDL, SOL, GAS) across experimental groups (CY, CO, A).

Muscle	Group	Endomisium	Perimisium	Epimisium	%
EDL	CY	+/-	++	+/-	10%
	CO	++	+++	++/+++	50%
	A	++	+/++	+	40%
SOL	CY	++/-	+/++	+++	60%
	CO	+++	+++	+++	50%
	A	++	+/++	+++	40%
GAS	CY	+/-	+/-	+	<10%
	CO	+++	+++	+++	70%
	A	+/-	+++	+++	40%

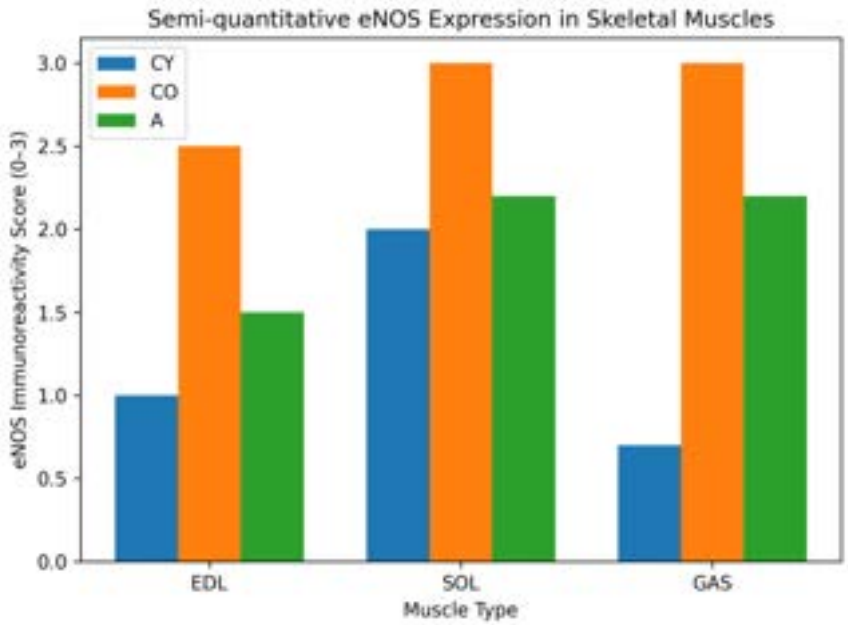

Figure 3. Graphic analysis of eNOS semi-quantitative immunoreactivity assesment in skeletal muscle types (EDL, SOL, GAS) across experimental groups (CY, CO, A).

In the EDL muscle, young control animals (CY) exhibited low eNOS expression, whereas a marked increase was observed in aged controls (CO). In the Aronia-supplemented group (A), eNOS levels were reduced compared to CO but remained elevated relative to CY group. In the SOL muscle, eNOS immunoreactivity was relatively high in CY animals and further increased in CO group. In contrast, the A group showed a moderate reduction compared to CO, approaching values closer to those observed in young controls. In the GAS muscle, minimal eNOS expression was detected in CY animals, followed by a substantial increase in CO group, representing the highest levels among all examined muscles. Aronia supplementation resulted in a noticeable decrease in eNOS expression; however, levels remained higher than in CY animals. Overall, aging was associated with a significant upregulation of eNOS immunoreactivity, particularly in glycolytic and mixed muscle types, while Aronia supplementation partially mitigated this effect.

Discussion

The present study demonstrates that aging is associated with a pronounced increase in eNOS immunoreactivity in skeletal muscle tissue, particularly in glycolytic (EDL) and mixed (GAS) muscle types, while supplementation with a functional beverage based on *Aronia melanocarpa* partially attenuated these alterations. These findings suggest that age-related vascular and oxidative changes in skeletal muscle are accompanied by adaptive modifications in nitric oxide signaling, which may be modulated by polyphenol-rich natural compounds (10). Nitric oxide plays a central role in skeletal muscle physiology through the regulation of vascular tone, blood perfusion, mitochondrial respiration, glucose uptake, and muscle regeneration. Under physiological conditions, eNOS-derived NO contributes to endothelial homeostasis and adequate oxygen delivery to metabolically active tissues. However, aging is characterized by progressive endothelial dysfunction, increased oxidative stress, chronic inflammation, and impaired mitochondrial activity, all of which can alter NO bioavailability despite elevated eNOS expression (10). Therefore, the increased eNOS immunoreactivity observed in aged control animals may represent a compensatory response to reduced NO efficiency and vascular impairment rather than improved endothelial function. The strongest age-related increase in eNOS expression was detected in the GAS muscle and, to a lesser extent, in the EDL muscle, both of which contain a substantial proportion of glycolytic fibers. Glycolytic muscles are known to be more susceptible to oxidative damage, mitochondrial dysfunction, and perfusion deficits during aging compared to oxidative muscles such as the SOL (11). The relatively high basal eNOS immunoreactivity observed in SOL muscle in young animals may reflect its higher capillary density, oxidative metabolism, and continuous postural activity. The additional increase in aged SOL muscle may indicate persistent endothelial activation associated with chronic age-related stress (11). Importantly, supplementation with *Aronia melanocarpa* juice resulted in a reduction of eNOS immunoreactivity in all examined muscles compared with aged controls. This effect was particularly evident in the GAS and SOL muscles, where immunoreactivity approached levels closer to those observed in young animals. These findings may indicate that the antioxidant and anti-inflammatory properties of aronia polyphenols reduce oxidative burden and improve endothelial homeostasis, thereby decreasing the need for compensatory eNOS upregulation.

Aronia melanocarpa fruits are exceptionally rich in anthocyanins, flavonoids, phenolic acids, and proanthocyanidins, compounds known to exert strong free radical scavenging activity and vascular protective effects. Polyphenols may improve endothelial function through several mechanisms, including reduction of reactive oxygen species, preservation of tetrahydrobiopterin (BH4), inhibition of inflammatory signaling pathways, and enhancement of NO bioavailability (12) Valera. Thus, the lower eNOS immunoreactivity observed after aronia supplementation may reflect improved enzymatic efficiency and restoration of physiological NO signaling rather than suppression of endothelial activity.

Conclusion

Polyphenol-rich *Aronia melanocarpa* juice demonstrates the capacity to modulate eNOS expression in aging skeletal muscles in a fiber-type dependent manner. The restoration of eNOS immunoreactivity, particularly in oxidative fibers, suggests improved NO bioavailability and vascular support in

aged muscle tissue. These findings indicate a potential myoprotective effect of functional polyphenol-rich supplementation and highlight eNOS as a key molecular target in strategies aimed at mitigating age-related skeletal muscle decline.

Acknowledgments

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Liposarcoma with extensive pelvic and thigh involvement: a case report

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Soft tissue sarcomas are rare malignancies in adults, with liposarcoma representing one of the most frequent histological subtypes. Their deep anatomical location and often indolent course may allow substantial growth before clinical detection, leading to presentation at an advanced stage. We present the case of a 65-year-old man with a gradually enlarging mass involving the right pelvic region and proximal thigh. Magnetic resonance imaging revealed a large, well-circumscribed, multilobulated lesion with mixed solid and cystic components and areas of fat signal intensity, extending longitudinally from the pelvis along the thigh. The mass demonstrated internal septations, heterogeneous enhancement, and diffusion restriction, while displacing adjacent pelvic organs without clear evidence of direct invasion. Based on imaging characteristics, a malignant soft-tissue neoplasm was suspected, with liposarcoma as the leading consideration. Histopathological analysis following core-needle biopsy confirmed a high-grade liposarcoma. This case highlights an uncommon presentation of a large liposarcoma spanning multiple anatomical compartments while maintaining relatively well-defined borders. It underscores the value of MRI in characterizing lesion extent, internal composition, and relationships with adjacent structures, which is essential for diagnostic orientation and preoperative planning. Recognition of such atypical and extensive manifestations is important for timely diagnosis, multidisciplinary management, and optimization of therapeutic strategy.
Keywords: *Liposarcoma; Magnetic resonance imaging;*

Introduction

Liposarcoma is one of the most common soft-tissue sarcomas in adults, accounting for 13–20% of all sarcomas with a preferential development in deep adipose rich compartments such as the thigh and retroperitoneum (1). Because these tumors often develop silently within deep fascial planes, they can reach large dimensions before presenting symptoms (2). Crossing over anatomical lines, they appear as masses that span multiple compartments, complicating both imaging interpretation and surgical planning (3). For the defining shape of liposarcomas, MRI is the imaging modality of choice. Key signs include heterogenous fat-signal intensity, thick internal septa, and areas of diffusion restriction, which helps differentiate malignant lipomatous tumors from benign lipomas and other lipomatous lesions (2, 4). Recent molecular studies have revealed unique genetic changes in various liposarcoma types. The well differentiated and dedifferentiated kinds feature MDM2/CDK4 amplification, while myxoid types hold certain fusion genes, providing both diagnostic markers and treatment targets (2). Despite advances in molecular diagnostics, large multicompartmental liposarcomas remains challenging; attaining negative surgical margins is crucial, as incomplete resection is associated with an increased risk of local recurrence (2). This case report presents an unusually large high-grade liposarcoma expanding across the right pelvis and proximal thigh, underscoring the essential role of MRI for accurate delineation, the integration of histopathologic and molecular data for definitive diagnosis, and the multidisciplinary approach required to optimise outcomes.

Case presentation

A 65-year male presented with a progressively enlarging mass involving the right gluteal region and proximal thigh. The patient reported delayed medical consultation despite gradual growth of the lesion over an extended period. There was no reported history of trauma, systemic symptoms such as fever or weight loss, or prior malignancy.

On presentation, a markedly large, palpable mass was noted involving the right gluteal and upper thigh region, with obvious asymmetry of the lower extremities. The lesion was non-tender on examination, with no overlying skin ulceration or signs of infection. Neurovascular status of the limb was not reported as compromised on initial assessment. Given the size and depth of the lesion, urgent cross-sectional imaging was performed.

Diagnostic imaging

MRI of the pelvis and right lower limb demonstrated a massive, septated, macrolobulated, relatively well-circumscribed solid-cystic lesion originating at the level of the right iliac crest and extending longitudinally into the distal third of the femur. The lesion measured approximately 20×19 cm in axial dimensions and 35 cm craniocaudally. It demonstrated mixed solid and cystic components with internal fatty inclusions, thick septations, heterogenous post-contrast enhancement, and diffusion restriction. A pelvic extension measuring 6×3 cm was present, causing mild mass effect with contralateral displacement of the urinary bladder and rectum without evidence of invasion. No ascites or osseous involvement was identified. Mild right inguinal lymph node enlargement was noted without suspicious morphology.

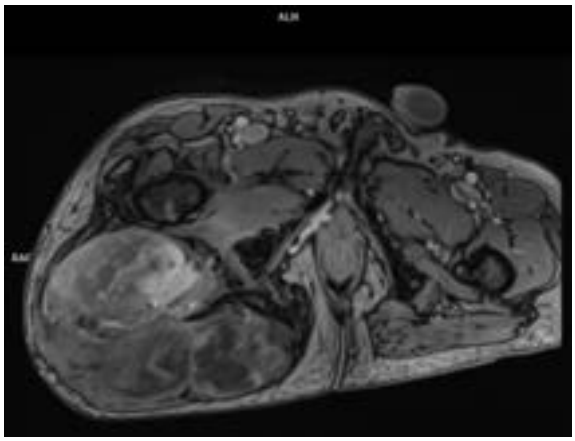

Figure 1. Contrast-enhanced T1-weighted MRI of pelvis – axial plane

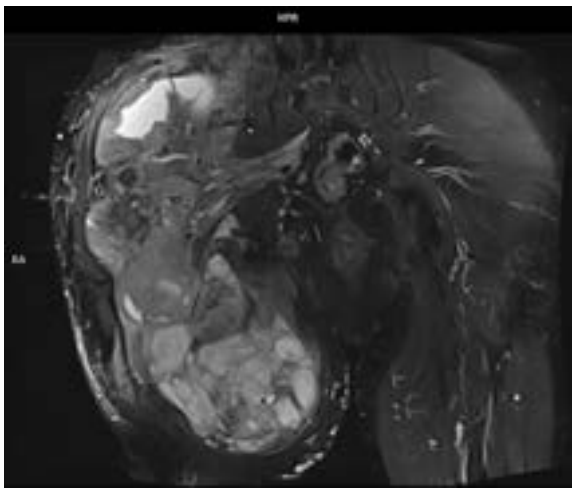

Figure 2. T2-weighted TIRM sequence of pelvis – coronal plane

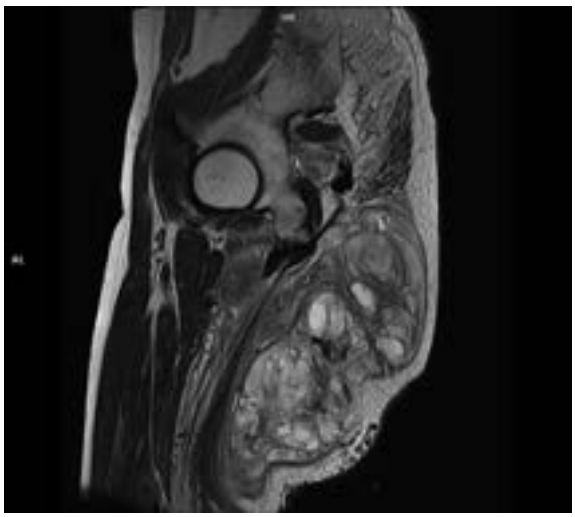

Figure 3. T2-weighted MRI of pelvis – sagittal plane

Discussion

Extensive liposarcomas that traverse the pelvis and thigh are rare but clinically important because of the following complex surgical planning. MRI reliably delineates tumor extent, internal heterogeneity and relationship to vital structures (5). The multilobulated, fat-rich, septated appearance in our patient mirrors patterns described in large retro-peritoneal liposarcomas. Histopathologically, the core-needle biopsy showed findings consistent with dedifferentiated liposarcoma, with strong nuclear expression of MDM2 and CDK4 supporting the diagnosis. These molecular hallmarks are characteristic of the 12q13-15 amplification seen in well-differentiated and dedifferentiated subtypes and are important diagnostic markers (2).

Surgical management must balance radicality with functional preservation. En-bloc resection of both pelvic and thigh components aims to achieve an R0 margin, aligning with data showing that negative margins are among the strongest predictors of overall survival in soft tissue sarcomas, regardless of size and grade (6). Adjuvant intensity-modulated radiotherapy (IMRT) allows for highly conformal dose delivery while sparing adjacent critical structures. Retrospective series of retroperitoneal and deep trunk sarcomas have reported that peri-operative IMRT, delivered at doses around 60Gy, is feasible and associated with favourable local control rates and low rates of severe toxicity. In these studies, improved local outcomes are often linked more strongly to surgical margin status than to radiotherapy variables alone (7).

Despite optimal multimodal therapy, dedifferentiated liposarcoma retains a higher propensity for late recurrence compared with well-differentiated disease, necessitating lifelong imaging surveillance; the SEER-based cohort reported a 5-year disease-free survival around 45% in dedifferentiated subtypes in population analyses (8). In this context, a coordinated, multidisciplinary approach that integrates precise imaging, molecular-guided pathology, aggressive surgery and tailored radiotherapy is considered essential to optimising outcomes.

Conclusion

The case underscores the pivotal role of detailed MRI assessment and molecular-driven histopathology in guiding surgical resection with curative intent for massive, multi-compartmental liposarcomas. Ongoing surveillance and emerging MDM2/CDK4-targeted therapies may become important in improving long-term disease control of this aggressive, yet initially indolent tumor.

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The impairment of glymphatic system as an undervalued element for the presence of prodromal sleep disturbances in Parkinson's disease

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Introduction: The glymphatic system is a recently described brain clearance pathway, with a critical role in metabolic waste clearance, particularly during slow-wave (N3) sleep. Sleep disruption may impair glymphatic function, facilitating the accumulation of proteins like alpha-synuclein, which is implicated in the pathogenesis of Parkinson's disease (PD). Importantly, sleep disturbances are among the earliest prodromal non-motor symptoms. **Aim:** To review existing literature and explore the relationship between the glymphatic system and prodromal sleep disturbances in PD. **Materials and methods:** A structured search was conducted using the PubMed and ScienceDirect databases, utilizing the following keywords: glymphatic system, Parkinson's disease, prodromal sleep disturbances, neuroinflammation and alpha-synuclein. **Results and discussion:** The glymphatic system facilitates cerebrospinal fluid exchange through perivascular pathways of the brain driven by sleep-dependent pulsatile mechanisms. Neuroimaging studies, including diffusion tensor imaging analysis along perivascular spaces (DTI-ALPS) suggest inefficient glymphatic flow in patients with prodromal PD. During this stage, patients experience reduced N3 sleep, which is exacerbated by sleep disorders like obstructive sleep apnea and insomnia. Reduced GS clearance promotes alpha synuclein aggregation and neuroinflammation, creating a vicious cycle. Therefore, sleep disturbances promote neurodegeneration in PD, and their early intervention may delay neurodegeneration. Future therapeutic directions include enhancement of cerebrovascular pulsatility via sleep-modulation, respiration techniques, body position, restoration of AQP4 polarization and astrocyte-targeted therapies. **Conclusion:** Understanding the pathogenetic links between prodromal sleep disturbances and the progression of PD through impairment of glymphatic system, provides prospects for prevention and early management of PD to improve the disease outcomes.

Keywords: *glymphatic system, Parkinson's disease, prodromal sleep disturbances, neuroinflammation, alpha-synuclein*

1. The glymphatic system

Although it has only been discovered 10 years ago, the brain's specialized network of micro vessels with the function of clearing neurotoxic waste is paramount and there is increasing curiosity to this physiology. The various physiological functions are immunity, transport of essential nutrients, and toxic waste clearance aid in maintaining brain homeostasis. The primary focus of this review is on the function of toxic waste clearance. The glymphatic system (GS) is a fluid-transport system travelling throughout regions in the brain, holding value in the maintenance of brain function and cognitive health.

Anatomical components of this system include: periarterial and perivenous spaces in the brain parenchyma.[1] A special component in the functioning of this network are the aquaporin-4 (AQP4) water channels (Figure 1).

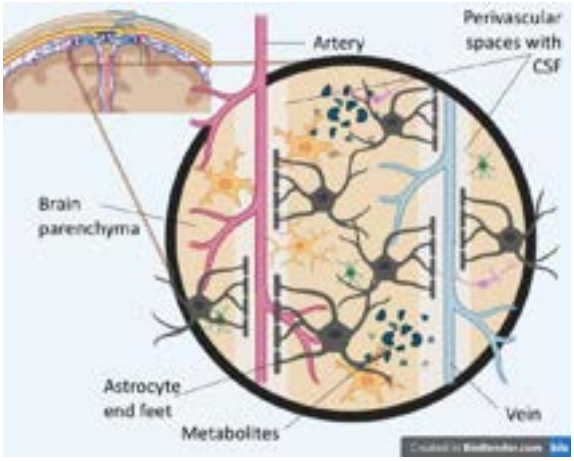

Figure 1. Anatomy of the glymphatic system Created in https://biorender.com

This drainage system consists of 3 functional parts, and are as follows:

The first being the glymphatic influx which occurs in the subarachnoid space. Cerebrospinal fluid (CSF) flows into the perivascular spaces within the brain parenchyma and bathes the penetrating arteries.[2] The fluid disperses via a polarised net fluid movement directed towards the perivenous and perineuronal space.[1] The second is the exchange of CSF and interstitial fluid (ISF). The CSF bulk flow is facilitated by the polarisation of AQP4 water channels, expressed at the astrocyte end-feet which lines the peri-arterial spaces. This facilitates the rapid movement of fluid between the perivascular space and the glial syncytium.[2] There are 2 types of aquaporins expressed in the brain predominantly, aquaporin 1 and AQP4. AQP4 being the main water channel in the parenchyma and the key component of glymphatic clearance.[2] Finally, the glymphatic efflux: the drainage of ISF into the perivenous space, from which metabolic waste from the ISF either re-enters the CSF or is drained out along the meningeal and cervical lymphatic vessels.[2]

1.1. Drivers of glymphatic flow

Ultrafast magnetic resonance encephalography (UMRE) revealed that pulsations associated to the respiratory cycle and changes in vasomotor tone also assist in glymphatic flow. Hence, disclosing that the main driving force of glymphatic flow is the arterial pulsations, originating from the cardiac systolic pressure wave.[1,2] There is increased GS function during sleep, which was demonstrated by doubling of CSF clearance rate, alongside 60% increase in interstitial space in brain during non-wake states as in comparison with wake states. Studies have shown that sleep is regulated by a sleep/wake cycle in which the clock gene plays a key role. The clock gene also known as CRY gene has indirect relations with glymphatic function by modulating the number of natural chances for glymphatic operation. Therefore, elements that cause disturbances in somnolence structure, like those seen in sleep-wake disorders may further encourage GS impairment.[3] Sleeping position can also manipulate the resistance of blood and CSF flow to the brain by the influence of gravity relative to the brain's vascular system. The right lateral position was proven to be more effective for GS clearance when researched in rats. Right lateral supine position during sleep reduces REM sleep time and increases slow wave sleep (SWS) in contrast to those who sleep in supine position.[4] Other ways to modulate and enhance quality of sleep are by sensory modulation which synchronizes the brain to surrounding rhythms via the adjustment of auditory, visual-auditory gamma, vestibular or olfactory stimulations.[4]

1.2. The importance of sleep in glymphatic system

Sleep is a vital element in the functioning of the GS. Evidence from a study showed a 90% decrease in the CSF influx in awake mice compared to those who were sedated.[5] New research advocates that glymphatic activity is significantly increased especially during Non-Rapid Eye Movement stage 3 (N3) SWS in contrast to wakefulness.[6] This is characterized by slow waves which range between 0.5 and 4.5 hertz on electroencephalography (EEG). As a result, pulsations are formed with a focus on the interval between 20–30 s as this reflects a time where vital physiological brain functions align with the electrical activity, which supports that waste clearance is mainly active during SWS.[7] An in vivo study conducted in humans, which used a Bold fMRI in coordination with an

EEG, discovered that large oscillations occurred during sleep every 20 s peaking around 0.05 Hz, resulting in a higher inflow of CSF showing signs of good glymphatic drainage.[7] Another study using fMRI also showed that slow wave activity (SWA) leads to slow oscillations in the cerebral blood volume, which impacts CSF bulk flow. A selective suppression of SWA demonstrated a rise in CSF amyloid beta levels the following morning suggesting a less efficient GS clearance upon lack of SWA. Thus, reinforcing the potential of a good quality sleep on the removal of neurotoxic waste solutes and expanding the benefits of sleep in the preservation of human health.[4]

Modern studies also support the association of greater sleep disturbances upon presence of the genetic variant rs724033 in AQP4, along with a reduced diffusion tensor imaging along the perivascular space (DTI-ALPS) index suggesting an indirect influence on GS clearance.[8]

1.3. Influence of impaired glymphatic system on neuroinflammation

GS and meningeal lymphatics together have an important role in neuroimmunology homeostasis by actively taking part in immune cell trafficking and clearance. Evolving studies propose various hypotheses on how significant amount of peripheral inflammation leads to neuroinflammation with the involvement of the GS. From one perspective, there are multiple systemic inflammatory processes in the body contributing to the accumulation of immune cells and their secretion of pro-inflammatory cytokines in the perivascular space which instigates local inflammation in the periphery of the brain. On the other hand, it is a vicious cycle between the aggregation of these inflammatory mediators which burden the glymphatic system and further perpetuates the inflammation. A mice study, which assessed alterations of the GS in response to lipopolysaccharide induced acute inflammation showed significantly reduced perivascular CSF flow.[9] Accumulation of immune cells, AQP4 depolarisation and reactive morphological changes in glial cells during concurrent response to inflammation contributes to reduced CSF exchange in the brain parenchyma which is followed by oedema. These are some of the proposed mechanisms by which chronic inflammation impedes glymphatic clearance and persists in a vicious cycle.[2] Thus, the GS dysfunction exists as being one of the potential drivers for chronic central neuroinflammation.

2. PD

Parkinson's disease (PD) is a progressive neurodegenerative condition resulting from degeneration of dopaminergic neurons in the substantia nigra pars compacta with the accumulation of misfolded alpha-synuclein.[10] PD is the second most common neurodegenerative disorder and its prevalence is expected to continue to grow significantly by 2050 mainly because of the increase in the aging population and extended life expectancy.[10] The clinical manifestations of PD gradually progress over a long period time, initially presenting with a prodromal stage characterized by non-motor symptoms.

3. Prodromal sleep disorders in PD

Sleep disturbances such as RBD, EDS, SDB, and OSA may precede years before the onset of motor symptoms, which is supported by a large-scale case control study assessing these manifestations over a period of 10 years. The general trend in the study was that the prevalence of the said non-motor symptoms increased in number with years closer to the actual diagnosis of PD in patients, which was not the same for the

controls. The frequency of the presence of sleep disorders was also always higher in patients with PD as opposed to the controls.[11]

3.1. RBD

REM-sleep behaviour disorder (RBD), is characteristically associated with significant REM sleep without atonia (RSWA), causing excessive motor activity during the period of dreaming. A type of RBD called isolated RBD (iRBD) seen in patients; it manifests on its own and resulting in GS dysfunction. Studies insist that iRBD is the strongest clinical marker of prodromal synucleinopathy.[12]

The pathophysiology of RBD is not well understood but gene mutations is a factor in recent discoveries. Various research focused on the genetic mutations linked to PD such as: SNCA, PINK1, LRRK2, Parkin, DJ-1, but particularly the single nucleotide polymorphisms (SNPs) in the Bone Marrow Stromal Cell Antigen 1 (BST1) gene has a significant relationship with motor deterioration and a risk factor for worsened disease progression in PD.[13]

The main clinical manifestations and symptoms that patients show are RSWA, dream-enactment behaviours being, emotional expression, verbalization and body movements during sleep, which can lead to injuries.[14,15]

A highly significant number of individuals with iRBD happen to develop PD as the relation between RBD and PD is based on neurodegeneration in REM sleep centres, namely the sublaterodorsal tegmental nucleus and the magnocellular reticular formation are affected, leading to reduced atonia.[14] This combination is clinically demonstrated with poorer motor and cognitive performance. REM sleep is especially important for drainage of neurotoxins in the brain and that happens via GS, however in RBD, the GS is disrupted and leads to a faster disease progression.[12] The ubiquity of RBD in PD patients is not steady and constant but increases as the disease progresses, relaying the idea that if a PD patient has not developed RBD yet, then they eventually will.[12]

The management for RBD involves the safety precautions according to the American Academy of Sleep Medicine (AASM). An evaluation should be made as to how severe the RBD is and is vital to assess if there's risk of harm to the patient and bed companion. Pharmacotherapies which can be beneficial for these patients are immediate-release melatonin; starting at a low dose for 5–7 days and a gradual increase made up to maximum dose being 12 mg per night.[16]

3.2. EDS

Excessive daytime sleepiness (EDS) is a prevalent symptom that is often indicative of underlying sleep disorders. According to the International Classification of Sleep Disorders – Third Edition, Text Revision (ICSD-3-TR), it is defined as the inability to maintain wakefulness and alertness during major waking episodes of the day, with sleep occurring unintentionally or at inappropriate times almost daily for at least 3 months.[17,18] Recent evidence suggests that EDS could be a primary aetiology before PD diagnosis.[19]

Patients with PD and EDS have been shown to present a reduced overnight decline in slow-wave activity. Radiopharmaceutical imaging studies focused on early-stage PD showed a correlation between dopamine transporter loss in the striatum, caudate nucleus, and putamen, and higher ESS scores. This suggests that dopaminergic nigrostriatal degeneration and the extension of neurodegeneration into the brainstem and hypothalamus[20], may contribute to the development of EDS in the early stages of PD.[19]

A meta-analysis study of EDS in PD patients demonstrated associations of EDS with older age and worse motor symptoms. Nevertheless, analysis with vPSG did not show a clear association with ESS scores, which suggests that various underlying factors may contribute to daytime sleepiness.[21] Some studies demonstrated a higher rate using ESS only, whereas others found no significant relationship in the data using both ESS and MSLT. Overall, the reported incidence rates of EDS in PD patients vary considerably, which could reflect differences in the results from subjective and objective assessments.[22] Thus highlighting the need to further explore the patients' self-reported sleepiness to consider potential treatment strategies and improve accurate diagnosis.

3.3. SDB

Sleep related breathing disorders include a spectrum of conditions commonly classified into Obstructive sleep apnea (OSA), Central sleep apnea (CSA), sleep related hypoventilation disorders, sleep related hypoxemia disorder and isolated symptoms such as snoring and catathrenia according to the ICSD-3-TR.[17] OSA is one of the most prevalent and severe SDBs with risks of multiple comorbidities, thus requiring more caution. The same is the case in PD. It is both a strong independent risk factor for developing PD due to factors including fluctuating respiratory muscle coordination, autonomic dysfunction and reduced respiratory drive; and a predictor of worse prognosis due to sleep fragmentation and intermittent hypoxemia which ultimately lead to the exacerbation of PD symptoms.[14] Therefore, early diagnosis of OSA using Apnea-hypopnea index (AHI) and vPSG to initiate treatment is of paramount importance in the management of PD. [23] This approach will result in better prognosis for patients presenting with both OSA and PD by decreasing the harmful effects of the mutually dysfunctional dynamic that persists between them. It is also, key to note that certain treatments can favour prognosis for one condition while exacerbating the symptoms of the other.[24]

3.4. RLS

Restless legs syndrome is a sensorimotor disorder also known as Willis-Ekbom syndrome, characterized by periodic leg movements during sleep. The prevalence of RLS is high in PD patients in which they experience an irresistible urge to move the limbs only providing a temporary relief. RLS is one of many potential sleep disorders present in early stage of PD.[25] There has been attempts to find a pathophysiological link between RLS and PD by brain imaging studies and functional imaging studies, however both lead to no clear evidence of any shared ideologies. In addition to that, there were also failed genetic association between RLS and PD. The updated treatment guidelines by AASM for RLS recommends the intake of gabapentin and iron replacement, and the use of levodopa and dopamine agonists have been discouraged.[26]

4. Relationship of glymphatic system & sleep disorders in PD

The function of the glymphatic clearance is impaired by several factors, one of the main ones being sleep dysfunction. Sleep disorders like RBD, EDS, SDB, RLS, and insomnia are some of the most common in patients with prodromal manifestations of PD. These disorders can present individually or in combination and decrease the quality of sleep by altering the structure of sleep. As a result, glymphatic clearance is affected since it is most effective during the N3 stage of sleep. This results in accumulation of toxic proteins like α-synuclein.

The outcome is a vicious cycle due to the aggregates, further driving GS dysfunction through obstruction of perivascular spaces and instituting a neuroinflammatory milieu in them and adjacent brain tissue. Thus, resulting in neurodegeneration, which leads to the full PD (Figure 2).

5. Benefits of research

Patients with neurodegenerative diseases have demonstrated an impairment of glymphatic clearance, identifying it as an area of required research. Thus, understanding this function offers new insights into the early prevention and progression of sleep disturbances in PD patients. Glymphatic imaging in the prodromal stage in PD patients could potentially serve as a non-invasive biomarker to monitor disease progression.

The in vivo assessment of the GS presents major challenges, encouraging the development of non-invasive imaging techniques. The DTI-ALPS index has recently become a more approved method. This method provides an indirect surrogate measure of the diffusivity of water along the deep medullary veins and glymphatic function in the brain. The use of DTI-ALPS as a biomarker could aid in monitoring therapeutic interventions such as sleep modulation techniques or pharmacological modulation of AQP4 and therefore facilitate a path for new disease modifying strategies in PD.[10] Prospective studies should aim to combine DTI-ALPS with fluid biomarkers, including alpha synuclein seed amplification assays in the CSF.[4] Furthermore, the potential use of robotics and artificial intelligence can assist in optimizing glymphatic flow by the adjustment in sleeping position and physical exercise could improve brain pulsations which has been established to facilitate glymphatic clearance.[4]

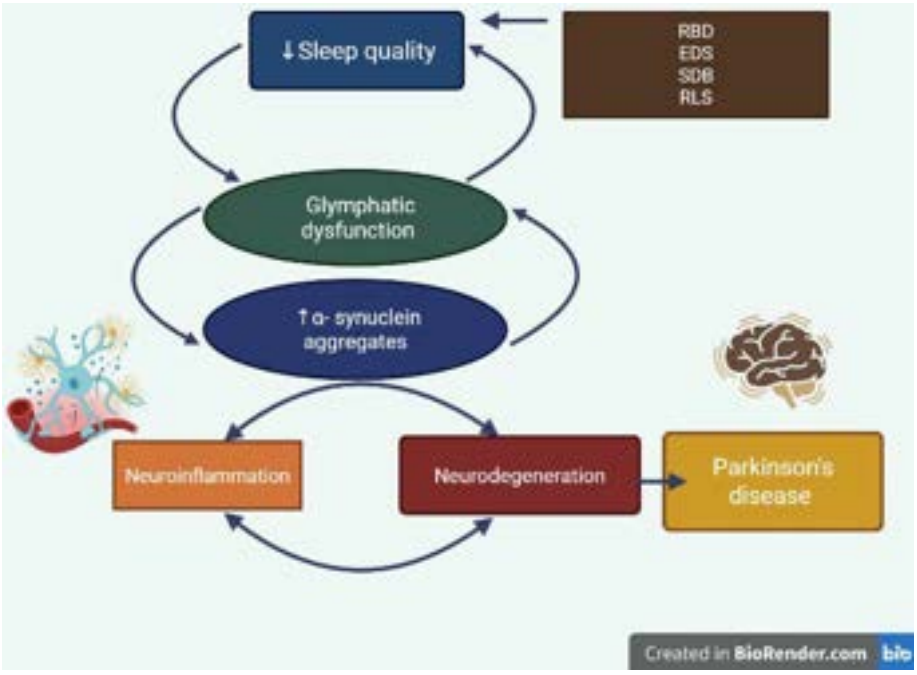

Figure 2. Implications of sleep disorders and glymphatic dysfunction in early PD.
Created in https://biorender.com
RBD: REM behavioural disorder, EDS: Excessive daytime sleepiness, SDB: Sleep disordered breathing, RLS: Restless leg syndrome

6. Conclusion

There is currently lack of clear understanding in the pathogenesis of PD in context to the presence of prodromal sleep disturbances in PD. The dysfunction of glymphatic system could be the bridge that explains the progressive pathophysiology and the reason behind this specific prodromal manifestation. The current evidence shows that the future of PD diagnosis and management should be focused on finding specific biomarkers that assess glymphatic dysfunction and enhancing glymphatic clearance along with appropriate management of sleep disorders to have a potential disease modifying effect.

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Thyroid-gut axis and the role of zonulin in autoimmune thyroid pathology

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Autoimmune thyroid diseases (AITD) are organ-specific disorders that may result in hyperthyroidism or hypothyroidism, although a significant proportion of patients remain in an euthyroid state for extended periods. The rising incidence of AITD, particularly chronic lymphocytic thyroiditis, necessitates a re-evaluation of its pathogenesis through the concept of the thyroid-gut axis. The gut microbiome serves as a fundamental factor in maintaining immune function and metabolic homeostasis. Intestinal dysbiosis and the associated increased permeability (often referred to as "leaky gut syndrome") may act as triggers for autoimmune processes within the thyroid gland. A key mediator in this mechanism is zonulin, a protein synthesized by the intestinal epithelium. Elevated zonulin secretion leads to the disassembly of tight junctions between epithelial cells, facilitating the translocation of antigens and endotoxins, which may provoke an autoimmune response in genetically predisposed individuals. Documented elevations in plasma zonulin levels among patients with AITD underscore its significance as a valuable diagnostic and therapeutic biomarker. Integrating insights into intestinal dysfunction into endocrinological practice offers an innovative approach to the prevention and management of autoimmune thyroid pathology.

Keywords: *AITD, zonulin, tight junctions, leaky gut syndrome*

Introduction

Autoimmune thyroid diseases (AITDs) are organ-specific diseases that can lead to hyper- or hypothyroidism, and a significant proportion of patients can remain euthyroid for a long period. The increasing incidence of AITDs, and more specifically chronic lymphocytic thyroiditis, requires a new interpretation of their pathogenesis through the concept of the thyroid-gut axis.

In recent years, convincing data have been accumulating that autoimmune diseases cannot be considered solely as a result of local organ damage, but as a consequence of complex interactions between genetic factors, immune dysregulation, endocrine mechanisms and changes in the intestinal microenvironment. In this context, the gastrointestinal tract is considered not only as a digestive organ, but as a central immunological and metabolic regulator, capable of modulating the systemic inflammatory response and autoimmunity.

The intestinal microbiome is a fundamental factor in maintaining immune function and metabolic homeostasis. Intestinal dysbiosis and the associated increased permeability ("leaky gut syndrome") could act as triggers for autoimmune processes in the thyroid gland. A key mediator in this process is the protein zonulin, synthesized by the intestinal epithelium. Its increased secretion leads to loosening of tight junctions between cells, facilitating the passage of antigens and endotoxins, which provokes an autoimmune response in genetically predisposed individuals.

Disruption of the integrity of the intestinal barrier and the subsequent abnormal antigen trafficking are considered as a potential pathophysiological mechanism for initiating and maintaining chronic autoimmune inflammation in thyroid diseases. In this regard, the "gut microbiome – intestinal barrier – immune system – thyroid" axis is emerging as a new concept in modern endocrinology and pathophysiology.

Understanding the mechanisms linking intestinal dysfunction to thyroid autoimmunity could open new opportunities for early diagnosis, individualized therapeutic approach, and prevention of autoimmune thyroid diseases.

Structure and function of the intestinal barrier.
Leaky gut syndrome

The intestinal barrier is a highly specialized dynamic system that separates the external environment from the internal environment of the body and plays a key role in maintaining homeostasis. Its main function is to selectively regulate the passage of nutrients, electrolytes and water, while simultaneously limiting the penetration of pathogenic microorganisms, toxins and antigens, protecting the macroorganism from excessive immune activation and systemic inflammation. In recent years, the intestinal barrier has been considered not only as a mechanical protective structure, but also as an active immunological and metabolic organ involved in the regulation of local and systemic immune responses.

The intestinal barrier is made up of four interconnected components – biochemical, microbiological, cellular and immune barrier. The biochemical barrier is formed by a mucus layer containing approximately 98% water, glycoproteins, mucin, secretory IgA (sIgA), and antibacterial substances produced by the normal intestinal microflora. This layer covers the epithelial cells and protects them from the harmful effects of the environment.

The cellular barrier consists of a single layer of epithelial cells connected to each other by tight junctions (TJs) and goblet cells that produce mucin (1, 2). TJs between enterocytes, which have long been considered static structures, have been shown to change their shape in response to various stimuli and are responsible for the proper permeability of the intestinal epithelium. These changes in TJs can be both physiological and pathophysiological (1).

Of particular interest are the molecular mechanisms that regulate paracellular transport across the intestinal epithelium. The discovery of zonula occludens toxin (Zot) and the subsequent identification of zonulin have greatly expanded our knowledge of the regulation of tight junctions and their role in the development of chronic inflammatory and autoimmune diseases.

Zot acts through a cascade of intracellular reactions that lead to protein kinase C (PKC)-dependent polymerization of actin microfilaments and subsequent alteration of TJs. Immunofluorescence has shown that Zot binding is differential in different parts of the intestine, being mainly found in the jejunum and distal ileum, but not in the colon. These differences in the small intestine suggest a different structure of the intestinal epithelial barrier and a difference in regional permeability (3). Beneath the enterocytes lies the lamina propria, which forms the intestinal immune system. It is composed of immune cells that form a lymphoid tissue called gut-associated lymphoid tissue (GALT) (2, 4). The GALT contains lymphocytes, Peyer's patches, lymphocyte clusters, and antigen-presenting cells (2). Intestinal macrophages are found in close proximity to the intestinal microflora and interact frequently with each other. This interaction is crucial for maintaining intestinal homeostasis and immune regulation (4). The gut microbiota plays a key role through the GALT, modulating both local intestinal and systemic immune responses. The GALT is crucial for establishing immune tolerance to self-antigens through Toll-like receptors (TLRs) in the intestinal mucosa (5).

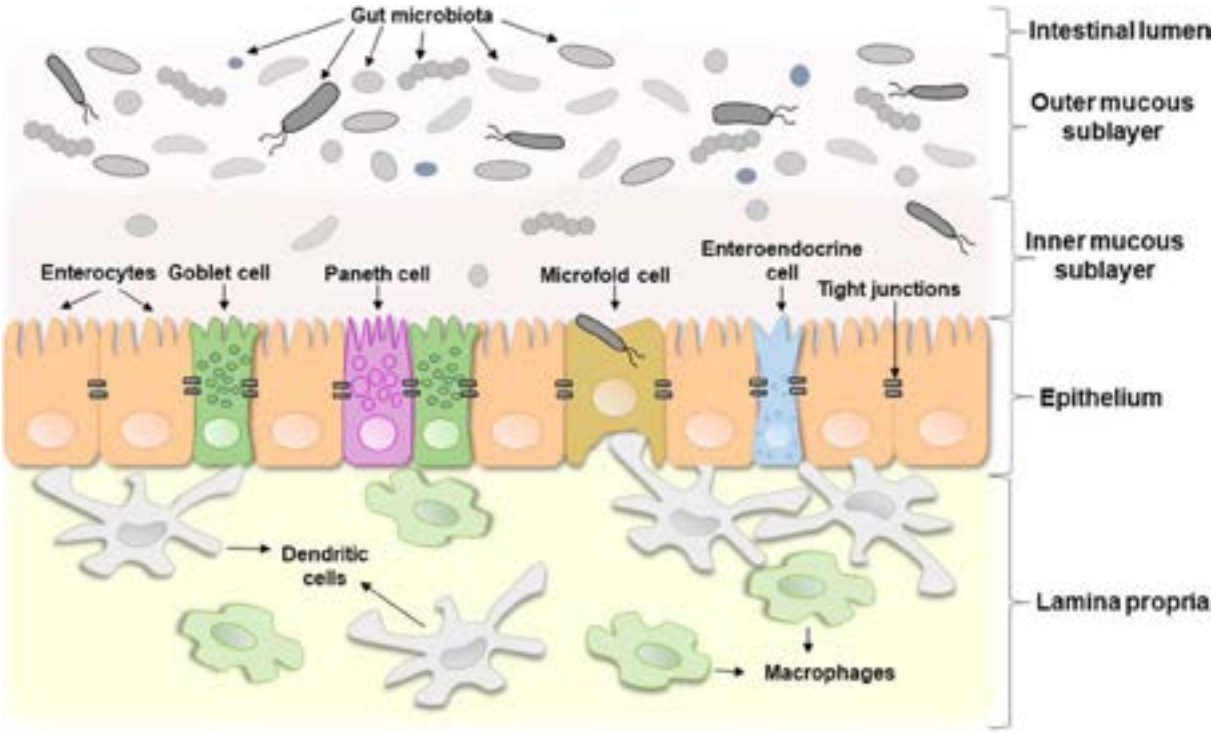

Figure 1. Structure of the intestinal barrier.

It is composed of four components: biochemical, microbiological, cellular and immune barrier. Disturbances in any of these components can lead to the development of numerous local and systemic diseases.

Impaired intestinal mucosal permeability, known as leaky gut syndrome, has recently been established as an important pathophysiological mechanism in the development of numerous chronic systemic diseases, including autoimmune diseases, obesity, allergic conditions, type 2 diabetes, depression, and cancer (1). Leaky gut syndrome is a condition characterized by the loosening of enterocyte tight junctions (TJs) and the abnormal trafficking of antigens and endotoxins (6). This condition may be influenced by the complex interplay between an individual's genetic predisposition, dietary habits, antibiotic use, and intestinal inflammation (7). Epithelial inflammation, followed by intestinal dysbiosis or dysbiosis, is a major part of the pathogenesis of this condition.

Among the most significant mediators of intestinal integrity disorders, the protein zonulin stands out, which in the last decade has emerged as a potential diagnostic and prognostic biomarker for leaky gut syndrome. Zonulin is secreted by epithelial cells and under physiological conditions is involved in the maintenance of normal intestinal permeability. When secreted, it binds to the epidermal growth factor receptor (EGFR), which leads to loosening of TJs. Microbial adhesion during bacterial overgrowth activates inflammatory signaling pathways through Toll-like receptors and zonulin signaling. The binding of zonulin to EGFR triggers a cascade in which phospholipase C activates protein kinase C-α, which leads to phosphorylation of ZO-1 and other target proteins. Ultimately, this causes actin depolymerization, disruption of tight junction integrity, and increased intestinal permeability with subsequent abnormal antigen trafficking (8).

Thyroid-gut axis considered as a potential link to autoimmune thyroid diseases

The gut microbiome is a complex ecosystem composed of trillions of microorganisms – bacteria, viruses, fungi and archaea, which play an essential role in maintaining metabolic and immune homeostasis. Under physiological conditions, the gut microbiota is crucial for the formation and proper functioning of gut-associated lymphoid tissue (GALT), as well as for the development of immune tolerance.

In recent years, experimental and clinical studies in humans and animal models have demonstrated that intestinal dysbiosis and inflammatory changes in the intestinal mucosa are observed in Hashimoto's thyroiditis. The hypothesis that primary changes in the gut microbiome and subsequent intestinal inflammation may be factors initiating thyroid autoimmunity is increasingly being discussed.

In addition to their ability to directly induce an inflammatory response through the activation of inflammasomes, gut bacteria also exert effects through their metabolites, including short-chain fatty acids (SCFA) and secondary bile acids, which can modulate the immune response and participate in the regulation of the thyroid-gut axis (6).

Of growing interest is the influence of diet on the gut microbiome and subsequent immunological changes. Dietary changes can lead to changes in the composition and metabolic activity of the gut flora, which in turn affects the production of bacterial metabolites with pro- or anti-inflammatory effects. These mechanisms are mediated by modulation of the macrophage M1/M2 profile and the balance between Th17 and Treg lymphocyte populations, which are essential for the development of autoimmune diseases (7, 11). Multiple studies have demonstrated a link between gut dysbiosis, increased intestinal permeability, and bacterial overgrowth in the development of Hashimoto's thyroiditis (6). A study by Demir et. al. found a statistically significant increase in zonulin protein levels in patients with Hashimoto's thyroiditis compared to controls (9). In another study, Tomov et al. did not find a significant association between zonulin levels, thyroid hormones, and autoantibodies, but reported increased zonulin levels in hypothyroid patients who were older, had more severe autoimmune thyroid disease, and had higher BMI. These results highlight the need for future studies with larger cohorts to more precisely elucidate the relationship between gut permeability and autoimmune thyroid pathology (12). Thyroid hormones exert multiple biological effects in the digestive system. T3 is directly related to the development and differentiation of intestinal epithelial cells. In hypothyroidism, gastrointestinal dysfunction is observed due to changes in gastrointestinal neuromotor function. A classic gastrointestinal symptom in hypothyroidism is constipation, but diarrhea as a symptom in patients with reduced thyroid function described in the study by Lauritano et. al. is associated with bacterial overgrowth and impaired neuromuscular function due to the developed hypomotility (6). Abnormal antigen trafficking stimulates the immune system and in patients with a genetic predisposition this could play a major role in the manifestation of AITD. Based on this mechanism of abnormal antigen trafficking and intestinal dysbiosis, Knezevic et al. discussed the thyroid-gut axis as a potential pathophysiological link to AITD (10).

Despite the accumulated data on the relationship between intestinal dysfunction and thyroid autoimmunity, it remains unclear whether the observed dysbiosis represents a primary etiological factor or a secondary

consequence of the autoimmune process. Nevertheless, the concept of the thyroid-gut axis is being established as a promising direction in modern pathophysiology and endocrinology, providing new opportunities for early diagnosis, prevention and targeted therapy in autoimmune thyroid diseases.

Conclusion

Increased intestinal permeability is emerging as a potential key element in the pathophysiology of AITD. The enterocyte-secreted protein zonulin represents a promising marker in the diagnosis of leaky gut syndrome. Integrating knowledge of intestinal dysfunction into endocrinological practice provides an innovative approach for the prevention and management of autoimmune thyroid pathology.

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Achievement of predicted maximal heart rate and ventilation during cardiopulmonary exercise testing in young athletes: the influence of sex, age and sport specificity

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Introduction: Cardiopulmonary exercise testing (CPET) is the gold standard for assessing the body's response to exercise. In children this method can diagnose conditions of the cardiovascular and respiratory systems, as well as evaluate overall functional capacity. **Aims:** The aim of this study is to compare the extent to which children participating in systemic sport can reach predicted maximal heart rate (HRmax) and predicted maximal ventilation (VEmax) during progressive CPET, as well as to examine the influence of age, sex and sport discipline. **Materials and methods:** Sixty children, aged 7–17, participating in systemic sport (30 swimmers and 30 track and field athletes) underwent symptom-limited progressive CPET using a modified Bruce treadmill protocol. HRmax and maximal minute ventilation (VEmax) were compared with age-specific predicted theoretical values. **Results and discussion:** Young athletes achieve a high percentage of their predicted HRmax during CPET (95.6 ± 3.9%). Track and field athletes achieved a higher percentage of predicted HRmax compared to swimmers (96.6 ± 3.7% vs. 94.5 ± 3.9%; p = 0.036). No difference was observed for achieved ventilation (87.7 ± 18.9% vs. 86.9 ± 14.0%; p = 0.84). In linear regression, sport discipline showed a trend towards higher HRmax in track and field athletes, while achieved ventilation appeared to be influenced more by age and sex. **Conclusion:** Sport discipline appears to influence heart rate response in young athletes, while ventilation seems to be largely independent and individual. These findings underscore the importance of developmental factors and sport specificity, when interpreting CPET results in children.

Keywords: *cardiopulmonary exercise testing, children, sport, heart rate, ventilation*

Introduction

Cardiopulmonary exercise testing (CPET) is the gold standard for assessing the integrated response of the cardiovascular and respiratory systems to exercise. (1) Recently, CPET has expanded to include the testing of the children for evaluation of the functional capacity. Accurate interpretation of CPET in children requires consideration of developmental factors, as physiological responses to exercise are strongly influenced by growth, maturation, and body composition. (2) In addition, participation in organized sport may induce training-specific adaptations that further modify exercise responses. (3) However, the extent to which different sport disciplines influence the achievement of maximal physiological responses in children remains incompletely understood.

Maximality criteria, such as the achievement of predicted maximal heart rate (HRmax) and ventilatory capacity, are commonly used. (4) While heart rate (HR) is often considered a reliable indicator of effort, ventilatory parameters may be more variable and influenced by non-training-related factors, particularly in younger populations. (2)

Aim

The aim of this study is to compare the extent to which children participating in different types of systemic sport (track & field and swimming) reach predicted HRmax and predicted maximal ventilation during progressive CPET, as well as to examine the influence of age, sex and sport discipline.

Materials and methods

This study was designed as a cross-sectional observational study evaluating exercise responses in children using symptom-limited CPET. Sixty children, aged 7–17, participating in systemic sport were included in the study. Participants were divided into groups according to the specific sport discipline they participate in – 30 swimmers and 30 track and field athletes. Main anthropometric characteristics of the participants are summarized in Tables 1–2.

Table 1. Main anthropometric characteristics of the participants

Variable	Track & Field	Swimmers	Total
Numbers of participants (N)	30	30	60
Age (years)	13.5 ± 2.5	11.9 ± 2	12.5 ± 2.3
Height (cm)	160 ± 0.1	160 ± 0.1	160 ± 0.1
Weight (kg)	49 ± 16.1	47.2 ± 10.5	48 ± 13.5
Body mass index (BMI, kg/m^2)	18.7 ± 3.6	18.4 ± 2.4	18.5 ± 3
Waist circumference (cm)	66.5 ± 9.3	66.27 ± 5.4	66.4 ± 7.5
Hip circumference (cm)	83.5 ± 10.4	81.9 ± 7.7	82.7 ± 9.1
Waist-to-hip ratio (WHR)	0.8 ± 0.04	0.81 ± 0.04	0.8 ± 0.04
VO_2peak (mL·kg^{-1}·min^{-1})	46.6 ± 6.2	46.5 ± 5.7	46.6 ± 5.9
HRmax (beats·min^{-1})	196 ± 7.7	192.2 ± 7.3	194.1 ± 7.7
MVV (L/min)	91.4 ± 33.3	85. ± 17.8	88.2 ± 26.7
HR reserve (%)	96.7 ± 3.6	94.5 ± 3.9	95.6 ± 3.9
Ventilatory reserve (%)	87.7 ± 18.9	86.8 ± 13.9	87.3 ± 16.5

Table 2. Distribution of participants according to gender

Category	Male	Female	Total
Track & Field	19	11	30
Swimming	17	13	30
Total	36	24	60

All participants were clinically healthy, with no known cardiovascular, respiratory, or metabolic disease and no acute illness at the time of testing. Written informed consent was obtained from parents or legal guardians prior to testing. The study was conducted in the Exercise Laboratory of the Department of Pathophysiology, Medical University of Plovdiv, under physician supervision.

CPET was performed on a motorized treadmill (h/p/cosmos, Germany) integrated with a Vyntus CPX system (Vyaire Medical, Germany), using a modified Bruce protocol adapted for children (Figure 1). The protocol consisted of 3-minute stages with progressively increasing workload, preceded by two low-intensity stages to allow gradual adaptation. (5)

Baseline pulmonary function was assessed by spirometry. Forced expiratory volume for 1 second (FEV_1) was measured and subsequently used to estimate maximal voluntary ventilation (MVV). Breath-by-breath gas exchange was measured using a calibrated metabolic cart, and HR was continuously monitored via ECG telemetry. (1) All equipment was calibrated daily according to manufacturer guidelines.

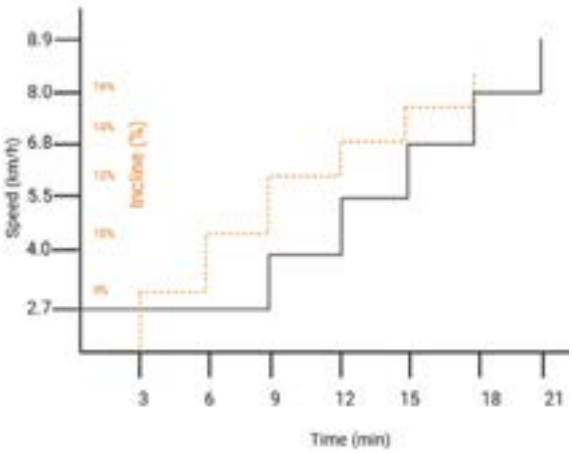

Figure 1. Stages of the modified Bruce treadmill protocol applied during CPET in pediatric participants.

Predicted HRmax was calculated using the Hunt equation (HRmax = 211 − 0.64 × age), and predicted MVV as FEV_1 × 35. (4) (6) Achieved values were expressed both as absolute values and as percentages of predicted maxima.

Statistical analysis was performed using Stata 18.0 (StataCorp LLC, College Station, TX, USA). Continuous variables are presented as mean ± SD. Normality was assessed using the Shapiro–Wilk test. Between-group comparisons were performed using t-tests or one-way ANOVA with Bonferroni post hoc correction where appropriate. Associations were explored using linear regression analysis with robust standard errors, including age, sex, BMI, and sport discipline as predictors. A p-value < 0.05 was considered statistically significant.

Results and discussion

Young athletes achieved a high percentage of their predicted HRmax during CPET, reaching on average 95.6 ± 3.9% of the theoretical HRmax. Track and field athletes demonstrated a significantly higher percentage of predicted HRmax compared to swimmers (96.6 ± 3.7% vs. 94.5 ± 3.9%; $p = 0.036$) (Figure 2). Although the difference was modest, it suggests a potential influence of sport-specific adaptations and testing modality. (7) Given that CPET was performed on a treadmill, the higher values observed in track and field athletes likely reflect greater familiarity with weight-bearing exercise compared to swimmers, whose training is performed in a non-weight-bearing environment. (4) (7)

Notably, several participants exceeded 100% of their predicted HRmax, highlighting the limitations of commonly used prediction equations in pediatric populations and supporting the interpretation of HR as an effort-dependent rather than absolute physiological marker. (8)

In contrast, achieved ventilation, expressed as a percentage of predicted maximal ventilation, did not differ between groups (87.7 ± 18.9% vs. 86.9 ± 14.0%; $p = 0.84$), with substantial variability and overlapping distributions observed in both groups (Figure 3). This suggests that ventilatory response is less influenced by sport discipline and more dependent on biological factors. (2) (7) Indeed, regression analysis indicated that achieved ventilation was more strongly associated with age and sex, which is consistent with the known effects of growth, lung development, and maturation on ventilatory capacity. (9)

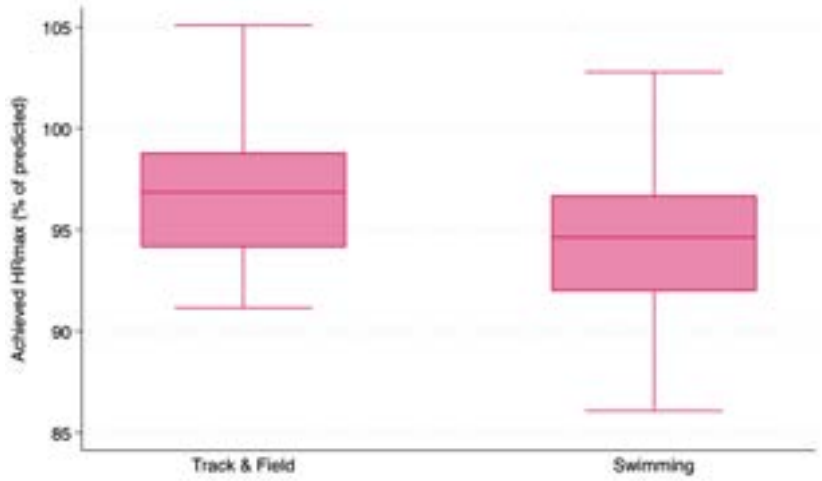

Figure 2. Achieved HRmax expressed as a percentage of predicted values in track and field athletes and swimmers.

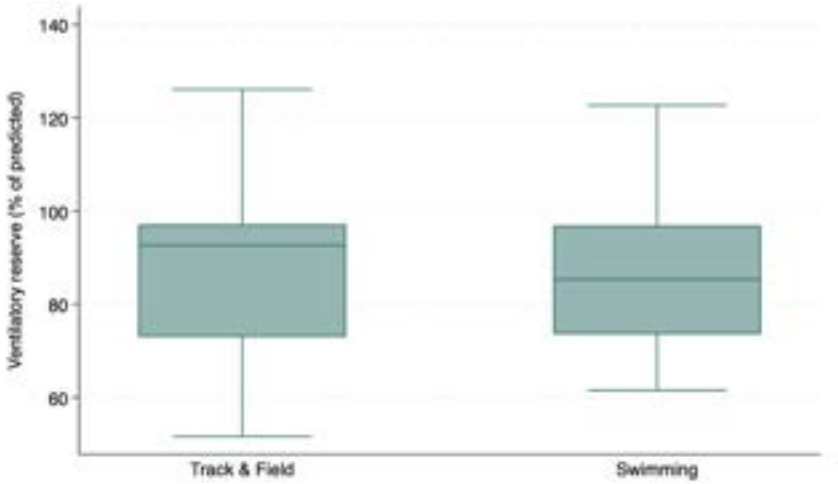

Figure 3. Ventilatory reserve expressed as a percentage of predicted MVV in track and field athletes and swimmers

Overall, these findings demonstrate that while young athletes are generally capable of reaching near-maximal HR during CPET, sport-specific differences are modest and primarily evident for cardiovascular responses. In contrast, ventilatory parameters appear to be more strongly determined by maturational factors rather than training modality, (2) (9) emphasizing the need for cautious interpretation of ventilatory indices as markers of maximal effort in pediatric populations. (2) (4)

Conclusion

Young athletes can achieve near-maximal HR values during treadmill CPET, supporting the use of HR as a practical indicator of exercise effort in pediatric populations. Sport discipline appears to modestly influence heart rate response, with higher achieved values observed in track and field athletes, likely reflecting both sport-specific adaptations and testing modality.

In contrast, ventilatory response does not differ between sport disciplines and appears to be largely determined by individual and developmental factors, including age and sex. These findings highlight the importance of considering both biological maturation and sport specificity when interpreting pediatric CPET results, particularly when using maximality criteria.

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Topographic anatomy and morphometry of the femoral nerve and its relationship to the femoral vessels

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Introduction: The femoral triangle is an anatomically and clinically significant region due to the presence of major neurovascular structures, including the femoral nerve, artery, and vein. Anatomical variations in the topographic relationships and morphometric characteristics of these structures constitute a potential risk factor for iatrogenic injury. **Aim:** The aim of the present study was to provide a detailed topographic and morphometric analysis of the femoral nerve within the femoral triangle. **Materials and methods:** The study was conducted through anatomical dissection of a lower limb fixed in a 10% formalin solution. The course, morphometric parameters and its topographic relationships to the femoral vessels were examined. Morphometric measurements were obtained using bony landmarks, namely the anterior superior iliac spine and the pubic tubercle. **Results:** The femoral nerve was located at a mean distance of 5.3 cm from the anterior superior iliac spine and 5.5 cm from the pubic tubercle. The mean length of the femoral nerve trunk within the femoral triangle prior to division was 2.3 cm. The femoral nerve occupied a lateral position relative to the femoral artery, while the femoral vein was situated medially. **Conclusion:** The present findings contribute to existing knowledge regarding the morphometry and topography of the femoral nerve within the femoral triangle and emphasize the clinical importance of neurovascular anatomical variability in this region. This data may assist in reducing the incidence of iatrogenic complications.
Keywords: *femoral triangle, femoral nerve, femoral artery, femoral vein, morphometry*

Introduction

The femoral nerve (FN) is the largest branch of the lumbar plexus, arising from the dorsal divisions of the ventral rami of L2–L4. After emerging from the lateral border of the psoas major muscle, it descends between the psoas major and iliacus muscles and passes beneath the inguinal ligament to enter the femoral triangle. Within this region, the FN typically divides into anterior and posterior divisions, giving rise to multiple motor and sensory branches that supply the anterior compartment of the thigh and the medial aspect of the leg (1). Due to its size, functional significance, and superficial location below the inguinal ligament, the main trunk of the femoral nerve is frequently accessed for femoral nerve block. Peripheral nerve blocks, including femoral nerve block, have become a widely accepted and effective method for providing postoperative analgesia, particularly in lower extremity surgery (2). However, the success and safety of this technique depend on precise knowledge of the topographic anatomy and morphometric characteristics of the femoral nerve in relation to surrounding structures. Given the close anatomical relationship between the femoral nerve and femoral vessels within the femoral triangle, the nerve is vulnerable to injury during arterial catheterization, venous cannulation, and other surgical or interventional procedures. Complications may arise from direct trauma or secondary compression due to hematoma or pseudoaneurysm formation. Iatrogenic injury to the femoral nerve can result in significant morbidity, ranging from sensory loss over the anterior and medial thigh to weakness or paralysis of the quadriceps femoris muscle and impaired knee extension (3). Despite the clinical relevance of this region, relatively few studies have focused on detailed morphometric analysis and anatomical variations of the femoral nerve within the femoral triangle. Moreover, variations in its branching pattern and spatial relationship to adjacent vascular structures may increase the risk of complications during femoral nerve block or vascular interventions (4). Therefore, a comprehensive understanding of the normal anatomy and its possible variations is essential for safe clinical practice.

Aim

The aim of the present study was to provide a detailed topographic and morphometric evaluation of the femoral nerve within the femoral triangle, including its relationship to key bony landmarks and adjacent vascular structures, to contribute to safer anesthetic and surgical procedures in this anatomically complex region.

Material and methods

The study was conducted through anatomical dissection of a lower limb fixed in a 10% formalin solution at the Department of Anatomy, Histology and Embryology in Medical University – Plovdiv. Upon dissection, the course, morphometric parameters and the topographic relationships of the femoral nerve to the femoral vessels were examined. Morphometric measurements were obtained using bony landmarks, namely the distance of the femoral nerve to the anterior superior iliac spine (ASIS) (Fig. 1); its distance to the pubic tubercle (PT) Fig. 2; the length of the femoral nerve trunk to its first branches (Fig. 3). A caliper was used for all measurements. Each measurement was carried out twice with an accuracy of up to 0.1 mm. The study procedure was approved by the Medical University – Plovdiv.

Figure 1. Distance of the FN to the ASIS

Figure 2. Distance of the FN to the PT

Figure 3. Length of the FN trunk before divisions

Results

The femoral nerve was located at a mean distance of 5.3 cm from the anterior superior iliac spine and 5.5 cm from the pubic tubercle. The mean length of the femoral nerve trunk within the femoral triangle prior to division was 2.3 cm (Table 1). The femoral nerve occupied a lateral position relative to

the femoral artery, while the femoral vein was situated medially.

Table 1. Morphometric measurements

	Distance to ASIS	Distance to PT	Length of femoral trunk
FN	5.3 cm	5.5 cm	2.3 cm

Discussion

The femoral nerve (FN) follows a well-described course from the iliac fossa into the femoral triangle, where it typically divides into anterior and posterior divisions after a short trunk (5). While numerous studies have focused on variations in the origin and formation of the FN within the lumbar plexus, morphometric data concerning its course and relationships within the femoral triangle remain relatively limited (6). Given its intimate anatomical relationship with the femoral artery and femoral vein, the FN is particularly vulnerable during surgical and interventional procedures involving these vessels (7). In the present study, the femoral nerve was located at a mean distance of 5.3 cm from the anterior superior iliac spine (ASIS) and 5.5 cm from the pubic tubercle. These findings are comparable to those reported by Pateliya et al., who documented a mean distance of 4.6 cm from the ASIS (8). Surface localization of the FN using palpable bony landmarks is of considerable clinical value, particularly for anesthesiologists performing femoral nerve blocks and for surgeons operating in the inguinal region. Accurate knowledge of these measurements may reduce the risk of iatrogenic nerve injury during blind or landmark-guided procedures. The mean length of the FN trunk within the femoral triangle prior to division in our study was 2.3 cm. This value is slightly greater than that reported by Pateliya et al., who observed a mean trunk length of 1.5 cm distal to the inguinal ligament. Importantly, previous authors have described early division of the FN above the inguinal ligament in a significant proportion of cases. Anloague and Huijbregts reported variations in 35.3% of lumbar plexuses, including division of the FN within the substance of the psoas major muscle (9). Other studies have documented early division in approximately 25% of specimens (10). Early or high division of the FN is clinically relevant, as it may result in incomplete femoral nerve block if not recognized. Variations in muscular anatomy or branching pattern may predispose the nerve to compression neuropathy. Although cases of the FN being embedded within the iliopsoas muscle have been reported, sometimes leading to diagnostic confusion during ultrasound-guided blocks, no such embedding was observed in our material (11). Overall, the coexistence of variations in the femoral nerve branching pattern and in the relationship and branching of the femoral vessels highlights the anatomical complexity of the femoral triangle. Since many procedures in this region are performed using landmark-based techniques, insufficient awareness of such variability may lead to preventable complications. Pre-procedural imaging, meticulous dissection during open surgery, and careful application of anatomical landmarks are therefore strongly recommended.

Conclusion

The present findings contribute to existing knowledge regarding the morphometry and topography of the femoral nerve within the femoral triangle and emphasize the clinical importance of neurovascular anatomical variability in this region. This data may assist in reducing the incidence of iatrogenic complications.

Abbreviations

FN – Femoral nerve; ASIS – Anterior superior iliac spine; PT – Pubic tubercle

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Breakfast consumption and glycemic control in children: effects on energy levels and cognitive function

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Introduction: Breakfast represents the first meal following the prolonged overnight fasting period and plays a critical role in the restoration of energy balance and metabolic homeostasis in children. Due to limited glycogen stores and high cerebral glucose demand, children exhibit increased metabolic sensitivity to prolonged fasting. Morning glycemic fluctuations have been associated with fatigue, reduced concentration, and impaired cognitive performance in school-aged children. **Aim:** This review aims to examine the relationship between breakfast consumption and postprandial glycemic control and their role in supporting energy metabolism and cognitive performance in children. **Materials and methods:** A narrative review of peer-reviewed scientific literature was conducted using major biomedical databases, focusing on studies assessing breakfast consumption patterns, glycemic characteristics of morning meals, and cognitive outcomes in pediatric populations. **Results and discussion:** The reviewed evidence indicates that the glycemic characteristics of breakfast meals, particularly glycemic index and postprandial glycemic stability, may influence attention, working memory, and information processing speed during the morning hours. Breakfasts with a lower glycemic index are generally associated with more stable glucose availability, which may support cognitive functioning and academic performance in school-aged children. However, existing findings remain heterogeneous, reflecting differences in study design, age groups, and cognitive assessment methods. **Conclusion:** The available evidence highlights the importance of breakfast quality and glycemic regulation as modifiable factors that may contribute to improved energy availability and cognitive functioning in children. Further research is required to clarify age-specific effects and to support the development of evidence-based nutritional recommendations.

Keywords: *breakfast consumption, glycemic control, cognitive performance, glycemic index, school-age children*

Introduction

Breakfast is the first meal following the overnight fast and plays a central role in restoring energy balance and metabolic homeostasis. Regular breakfast consumption has been associated with improved performance in attention, executive function, and memory, whereas breakfast omission has been linked to adverse metabolic and functional outcomes (Adolphus et al., 2016). Despite its recognized importance, breakfast is frequently skipped among children and adolescents, with reported rates of 20–30% (Vereecken et al., 2009; Deshmukh-Taskar et al., 2010; Hoyland et al., 2012). Evidence suggests that both breakfast consumption and its glycemic characteristics influence cognitive performance by modulating postprandial glucose stability (Micha et al., 2010; Cooper et al., 2012). This is particularly relevant in children, whose brain accounts for a substantial proportion of total glucose utilization while hepatic glycogen reserves are limited following overnight fasting.

Aim

To examine the relationship between breakfast consumption and postprandial glycemic control, and their role in supporting energy metabolism and cognitive performance in school-aged children.

Methods

A narrative review of peer-reviewed scientific literature was conducted using PubMed, Web of Science, and Scopus databases. Keywords included “breakfast consumption”, “glycemic index”, “glycemic control”, “school-age children”, and “cognitive performance”. Peer-reviewed clinical studies, randomized controlled trials, systematic reviews, and longitudinal cohort studies published in English were included.

Results

Physiological basis of glycemic regulation

Blood glucose homeostasis is maintained through the opposing actions of insulin and glucagon on hepatic metabolism (Bang et al., 2023; Bivolarska, 2025). Glucagon activates the cyclic adenosine monophosphate – protein kinase A (cAMP-PKA) signaling cascade, promoting glycogenolysis and suppressing glycogen synthesis, while insulin reverses these effects in the postprandial state (Bivolarska, 2025). Glucagon additionally exerts insulinotropic effects via glucagon-like peptide-1 (GLP-1) receptor activation on beta-cells, contributing to fine-tuned glucose regulation (Bethea et al., 2021). Cerebral glucose is transported across the blood-brain barrier via glucose transporter 1 (GLUT1) and taken up by neurons via glucose transporter 3 (GLUT3), undergoing glycolysis and mitochondrial oxidation to generate adenosine triphosphate (ATP) for synaptic transmission and neurotransmitter synthesis (Petersen & Shulman, 2018; Muraleedharan & Dasgupta, 2021). In children, cerebral glucose utilization is disproportionately high relative to hepatic glycogen reserves, rendering them particularly sensitive to morning glycemic fluctuations (Adolphus et al., 2013).

Effect of breakfast skipping on blood glucose

Breakfast omission prolongs the overnight fasting state, sustaining elevated glucagon, cortisol, epinephrine, and ghrelin with suppressed insulin and adiponectin – collectively promoting hepatic glycogenolysis and peripheral insulin resistance (Adolphus et al., 2016; Bivolarska, 2025). At the cellular level, fasting activates AMP-activated protein kinase (AMPK), a master metabolic sensor that redirects substrates toward energy production rather than storage (Muraleedharan & Dasgupta, 2021). Population data indicate that 20–30% of children regularly skip breakfast, with higher prevalence among females, older adolescents, and socioeconomically disadvantaged groups (Sincovich et al., 2022). Progressive hepatic glycogen depletion increases glycemic variability and is associated with exaggerated postprandial glycemic excursions following the next meal (Adolphus et al., 2016; Sunram-Lea et al., 2021).

Breakfast composition and glycemic index

The glycemic index (GI) and glycemic load (GL) of breakfast carbohydrates are primary determinants of postprandial glucose dynamics (Sunram-Lea et al., 2021). High-GI foods generate rapid glucose peaks followed by compensatory insulin surges and reactive hypoglycemia, while low-GI foods produce gradual, sustained glucose increments (Cooper et al., 2012; Adolphus et al., 2016). In a randomized crossover trial, peak blood glucose was significantly higher following a high-GI breakfast (7.01 vs. 6.46 mmol/L), with a greater incremental area under the curve (116.6 vs. 80.9 mmol/L × 120 min) (Cooper et al., 2012). Substituting sucrose with the low-GI disaccharide isomaltulose reduced the 3-hour postprandial glycemic area by 48% and extended stable glucose availability by over 35 minutes in children aged 5–7 years (Sunram-Lea et al., 2021).

Meal timing and circadian regulation

The timing of food intake interacts directly with the endogenous circadian system. Early morning breakfast activates the CLOCK:BMAL1 (Circadian Locomotor Output Cycles Kaput:Brain and Muscle Arnt-Like 1) complex, upregulating glucose transporter 4 (GLUT-4), GLP-1 secretion, and hepatic glycogen synthesis via glycogen

synthase 2 (GYS2), optimizing glucose processing efficiency (BaHammam & Pirzada, 2023). Delayed meal timing disrupts peripheral circadian rhythms: the plasma glucose circadian rhythm is delayed by approximately 5.7 hours when meals are shifted later (Kaneda et al., 2025). Children exhibit a morning chronotype aligned with early food intake, which shifts toward eveningness during puberty, contributing to breakfast skipping and circadian misalignment (Ricotti et al., 2021). A systematic review of over 286,000 children demonstrated that breakfast skipping was associated with overweight, insulin resistance, elevated blood pressure, and metabolic syndrome (Ricotti et al., 2021).

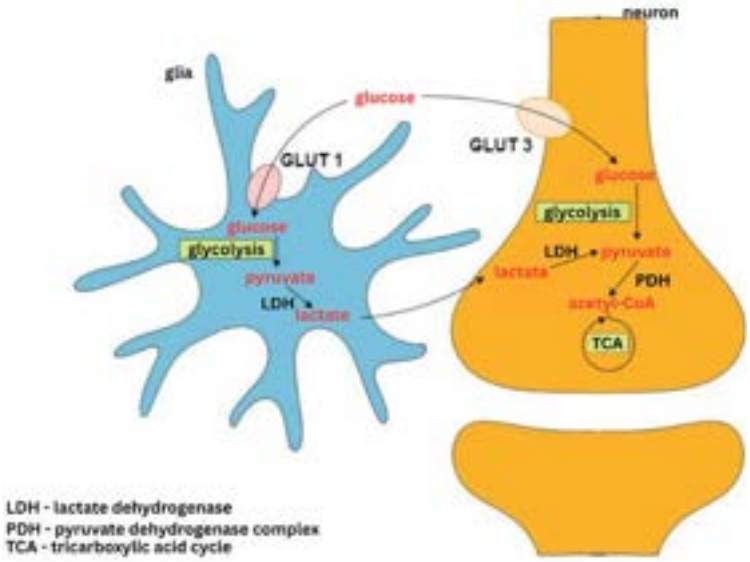

Figure 1. Carbohydrate metabolism in nervous tissue (neurons) (Bivolarska, 2025)

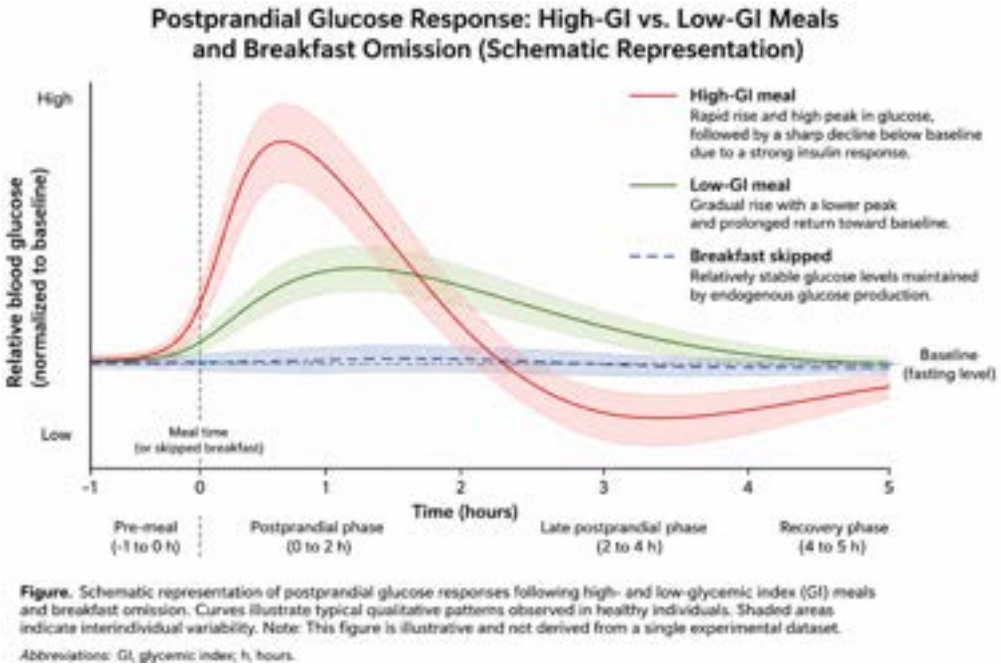

Figure 2. Schematic representation of postprandial glucose responses following high- and low-glycemic index (GI) meals and breakfast omission. Curves illustrate typical qualitative patterns based on published data (Cooper et al., 2012; Sunram-Lea et al., 2021). Figure created with AI assistance.

Effect on energy levels

Following a high-GI breakfast, the hyperinsulinemic response typically reduces blood glucose to or below fasting levels within 2–3 hours – precisely during peak academic demand. Postprandial sleepiness is mediated through insulin-driven tryptophan transport, increasing hypothalamic serotonin availability and suppressing orexinergic arousal signaling (Kaneda et al., 2025). Children who skip breakfast exhibit a progressive glucose decline associated with fatigue, reduced alertness, and slower reaction times (Adolphus et al., 2016). Physical activity contributes synergistically through insulin-independent GLUT-4 translocation in skeletal muscle: brief movement breaks of 2 minutes every 20 minutes significantly attenuate postprandial glucose peaks as demonstrated in sedentary adult populations (Kaneda et al., 2025). A 7-day crossover trial demonstrated that breakfast consumption reduced morning hunger and attenuated compensatory carbohydrate intake later in the day (Zakrzewski-Fruer et al., 2021).

Breakfast and cognitive function

Stable postprandial glucose supports ATP synthesis, acetylcholine production for cortical arousal, and dopaminergic prefrontal signaling via dietary tyrosine (Adolphus et al., 2016; Bivolarska, 2025). Among 21 acute studies comparing breakfast with fasting, 13 demonstrated positive attentional effects (Adolphus et al., 2016). A randomized controlled trial confirmed significant improvements in reaction time and sustained attention following breakfast consumption, with effects greatest in children with lower baseline performance (Adolphus et al., 2021). Post-breakfast subjective concentration ratings were significantly higher compared to fasting ($p = 0.0012$) (Adolphus et al., 2021). Low-GI breakfasts demonstrated attentional advantages in 6 of 9 comparative studies, with benefits accumulating progressively across the morning (Cooper et al., 2012; Adolphus et al., 2016).

Memory consolidation is highly dependent on hippocampal function and its sensitivity to glucose availability. Breakfast provides the postprandial glucose increment necessary to support hippocampal neuroenergetics, insulin-mediated synaptic plasticity, and acetylcholine synthesis in memory circuits (Adolphus et al., 2016). Among 15 acute memory studies, 8 demonstrated positive effects of breakfast relative to fasting, with visual-spatial memory – a predictor of reading comprehension and academic achievement – most consistently facilitated (Adolphus et al., 2021). A six-year longitudinal cohort study of 511 children demonstrated that regular breakfast consumption at age 6 was associated with gains of 5.54 points in verbal IQ and 4.35 points in full-scale IQ at age 12 (Liu et al., 2021).

Breakfast composition influences behavioral regulation through neurochemical pathways. Carbohydrate ingestion drives insulin-mediated tryptophan transport, increasing central serotonin synthesis and supporting mood stabilization and reduced aggression (Davidson et al., 2022; Tani et al., 2023). Protein provides tyrosine for dopaminergic impulse control in prefrontal circuits (Bivolarska, 2025). A 7-day prospective study of 281 Japanese school children found that frequent grain dish consumption at breakfast was independently associated with significantly fewer behavioral problems (coefficient = -12.2, 95% CI: -22.8 to -1.58), while confectionery-based breakfasts showed no protective effect (Tani et al., 2023).

Practical recommendations

The optimal breakfast for school-aged children combines low-GI carbohydrates

(GI less than or equal to 55), protein at 13–16% of energy, and adequate fat and fiber to modulate gastric emptying and glycemic absorption rate (Micha et al., 2011; Adolphus et al., 2016). Consumption within two hours of waking aligns food intake with peak adiponectin and cortisol activity and maximal CLOCK:BMAL1-driven metabolic efficiency (BaHammam & Pirzada, 2023). Multi-level school breakfast programs combining free provision, nutrition education, and parental engagement are most effective in reducing skipping prevalence and improving dietary quality (Ricotti et al., 2021). Glycemic stability is further supported by structured movement breaks and adequate sleep, which enhances insulin sensitivity and restores adiponectin and cortisol rhythmicity (BaHammam & Pirzada, 2023; Kaneda et al., 2025).

Discussion

The reviewed evidence consistently supports a causal link between breakfast consumption, glycemic stability, and cognitive performance in school-aged children, with effects operating across multiple physiological levels – from hormonal regulation and cellular energy sensing to neurotransmitter synthesis and circadian clock entrainment. The glycemic quality of breakfast – rather than caloric content alone – emerges as the primary modifiable determinant of morning metabolic and cognitive function.

Several limitations of the existing literature warrant acknowledgement. Heterogeneity in study design, breakfast manipulations, and cognitive assessment tools limits direct comparison across studies. Most acute studies assess outcomes at a single postprandial time point, potentially missing the temporal dynamics of glycemic effects. The mechanisms linking specific macronutrients to discrete cognitive outcomes require further elucidation through controlled pediatric trials incorporating continuous glucose monitoring and neuroimaging. Age-specific nutritional thresholds and the interaction between breakfast composition and individual metabolic variability – including chronotype, gut microbiome, and insulin sensitivity – represent priorities for future research (Adolphus et al., 2016; BaHammam & Pirzada, 2023).

Conclusion

Breakfast quality and regularity constitute central modifiable determinants of metabolic stability, energy regulation, and cognitive function in school-aged children. Low-GI breakfasts with adequate protein, consumed within two hours of waking, support stable neuroenergetic conditions underpinning sustained attention, memory consolidation, and behavioral regulation throughout the school morning. Longitudinal evidence confirms that these acute advantages accumulate into measurable developmental benefits, including higher IQ scores and improved academic achievement. Evidence-based strategies targeting breakfast composition and regularity – through parental education and school breakfast programs – represent effective approaches to improving cognitive and health outcomes in pediatric populations.

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Principles and practice in the use of the maximal lactate accumulation rate (VLamax) for athletic performance assessment

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Introduction: The maximal lactate accumulation rate is a physiological metric which shows the rate at which lactate levels can change acutely. This is theoretically a surrogate measurement for the glycolytic capacity of muscles, and especially in those where type IIx fibers are abundant. **Aim:** The aim of this review is to compile all the up-to-date information on the practical use of this metric and its effectiveness in performance assessment. **Methods and materials:** The medical databases Scopus, Web of Science, PubMed and Google Scholar were searched for the purpose of writing this review article. **Results and discussion:** The vLamax calculation involves performing a maximal intensity physical test of a specific duration. This test can be done in several different ways, the most often used being a cycling ergometer test. The duration of the test is also under scientific scrutiny and different studies suggest ranges from 10 to 30 seconds. This is mainly inspired by the theoretical basis of muscle metabolism, which suggests that maximal glycolytic capacity is reached between 3 and 30 seconds of maximal effort, after the use of phosphocreatine stores and before the full activation of oxidative metabolism. Studies show that vLamax can reliably predict performance for explosive sprint-type sports lasting 15–30 seconds. **Conclusion:** vLamax is a practically useful metric for assessing performance due to its simplicity and time-efficiency. Still as of now this is limited to explosive sports of limited duration. Further investigation is warranted in its role in estimating medium-intensity and endurance performance.

Keywords: *maximal lactate accumulation rate, performance, glycolytic capacity, explosive sports*

Introduction

Athletic performance and career longevity is one of the ultimate goals of the exercise and sports medicine departments of every athlete's team. To this end pre-competition assessment and performance analysis are crucial parts of the competitive season. Most commonly this is done with procedures like the cardiopulmonary exercise test (CPET) which is a comprehensive and detailed way to investigate the metabolism and aerobic capacity of athletes. This however might not be enough for certain sports. Aerobic capacity is important in various endurance and intermittent sports, but explosive ones like sprinting, weightlifting and even certain aspects of cycling are left behind because metabolic assessment of such athletes has not been universally accepted or popularised. One of the latest advances in metabolic testing has been the measurement of the maximal lactate accumulation rate (VLamax). It consists of a maximum intensity test which actively triggers the non-aerobic pathways for energy generation, paired with measuring blood lactate levels and how they change in time. The idea for such a test is not new and has been in development and use for more than 40 years. However, it has been somewhat unintentionally 'gatekept' from the international sport science community because of the origin of its pioneers – Germany (1). This led to publications written solely in German which were inaccessible to English speaking scientists and thus the concept was not largely popularised. The ideologist of the VLamax idea, along with colleagues, developed further the concept according to the biochemical understanding of muscle metabolism. Slowly the idea also gained traction outside of Germany and is now widely used around the globe as a test for the anaerobic power of athletes. Still it has not fully been standardised and different schools of mind in sport science developed and use the method in different ways, which

has sparked the need for standardisation and acceptance on various points of argument that still remain (2).

Materials and methods

The relevant medical databases PubMed, Scopus and Web of Science were included in the literature search, which was undertaken up to April 2026. Various combinations of the terms ‘lactate’, ‘accumulation’, ‘rate’, ‘muscle’, ‘metabolism’, ‘athletic’, ‘performance’ were used as search words. In total 22 articles were included in the final manuscript.

Basics of muscle metabolism

The metabolic processes seen in muscles that yield much needed ATP can be separated into three main pathways: phosphagen, glycolytic and aerobic (3,4). Each of these pathways is activated on a different intensity scale and has a more or less a well-defined duration through which it is the main cellular fuel. Indeed the metabolic switch that determines which pathway is the most active is the duration and intensity of exercise (5). Generally each of them is activated in sequence, starting always with the phosphagen system. In the beginning of exercise the first ~3.5–10 seconds are supplied with energy from readily available ATP and rapidly resynthesised ATP through the action of creatine kinase. The capacity of this system is quickly diminished, which requires the activation of the next pathway – that of glycolysis. Since its main product is lactate, this path is also called ‘lactic’, as opposed to the phosphagen, which is called ‘alactic’ (6). This metabolic route is quickly activated after the depletion of the previous one. Since anaerobic glycolysis is not an efficient ATP generator, producing only 2 ATP molecules per each glucose molecule, this route also rapidly can become inadequate in supplying the required amount of ATP. This is the reason that it can only contribute to a maximum of a minute of continuous exercise. After that the aerobic pathway becomes the main supplier of ATP through the oxidative phosphorylation process and the breakdown of glucose and fatty acids.

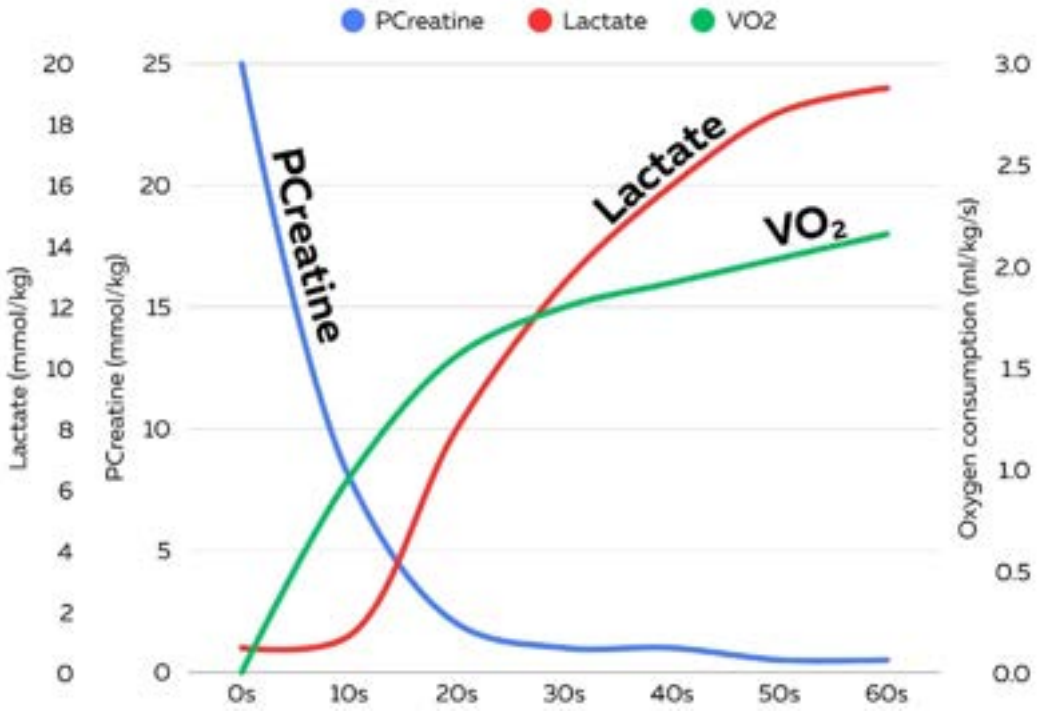

Figure 1. Temporal Dynamics of Energy Metabolism in Exercise.

All these pathways are of course simultaneously contributing to energy delivery, where there is no literal switch between them that completely inactivates one (7). Rather the intensity and duration of exercise determine which of the three is going to be the main supplier of energy in that moment. Thus they cannot be measured separately, but still in sport science assessing the relatively individual capacity of each pathway is useful for performance analysis.

Laboratory assessment of metabolic pathways

Arguably the most popular way to measure the metabolic efficiency of an athlete is to conduct a cardiopulmonary exercise test (CPET) with gas analysis. This method is a gold standard globally for measurement of the aerobic capacity of athletes and has been so for more than half a century, providing endurance athletes with a reliable and practically essential analysis (8, 9). A standard CPET provides somewhat direct metabolic data in the form of consumed oxygen and expired carbon dioxide, respiratory quotient, ventilatory thresholds of intensity, all of which can be used to precisely analyse the aerobic metabolic efficiency of an athlete. Contrary to this, tests for anaerobic metabolism for a long while did not provide direct metabolic data. Traditionally they can measure either power output, oxygen deficit and debt and a combination of the metrics. Measuring oxygen debt after exercise is the closest metabolic marker for anaerobic capacity, but still remains indirect, inconclusive and dependent on confounding factors (10).

The test for maximal lactate accumulation rate, also known most commonly as VLamax, has been one of the latest advancements in metabolic anaerobic testing in the last 15–20 years. It was first proposed as a concept by Mader and colleagues in the 1980s, but remained in usage mostly in the German sport science community, since studies were published in German (1). It was later popularised by prominent papers in English by Heck et al. (4), which sparked more interest in the international sport science community. The advancement in lactate testing equipment also accelerated VLamax research, as it became more accessible and popular. So much so that we have reached a point in which numerous studies have been published on the topic, describing different protocols and different calculation methods, sparking the need for a more unified approach and standardisation, if this metric is to be applied in everyday practice. A few systematic reviews have been published regarding this topic, which explore in detail the theory behind most recommendations for practical application, so this will not be discussed here and the reader is pointed to these articles for detailed reading (2, 11).

Practical aspects of VLamax testing

Typically VLamax calculation requires the following procedures: blood lactate level measurements done before the test at rest and after the test on several occasions, following criteria, described later; a short duration maximum effort exercise on an ergometer.

The first aspect of testing the lactate accumulation rate is to instruct the athlete to not partake in any high intensity fatiguing training at least 72 hours before the test, ideally at least 4 days, since the test itself consists of a maximal intensity effort (12). Therefore a risk is taken if the athlete performs the test already in a fatigued state. The best-case scenario will be a suboptimal test result, while the worst-case scenario can be an injury.

Another aspect is the nutrition before such a test. Since the metabolic system that is specifically tested here is the glycolysis

pathway, carbohydrate loading before such a test is advisable, since it will lead to fuller glycogen stores in the muscles and better expected results (13, 14). On the contrary, low-carb diets impair this aspect of performance, so are not advisable.

The next step of testing requires a choice of adequate testing modality, specifically what type of ergometer device to be used. Typically every athlete develops specific biomechanical efficiency, related to the sport they practice (15), so it is advisable to use the corresponding device to the sport modality, e.g. cyclist – cycling ergometer, runner or football player – treadmill, etc. (2, 11). The chosen device will also determine specifics like the duration of the test, which falls on average between 10 and 30 seconds.

Most commonly, a cycling ergometer is chosen for VLamax testing, part of the reason being its popularity in sports laboratories. As a result most research is also done using cycling ergometers, which has given us a lot of evidence regarding what is the best practice for this modality.

If cycling is chosen as the test modality some practical aspects are: test duration should be in the range of 10–15 seconds, where once a duration is chosen, it should be applied strictly especially if tests are performed continuously and used for progress development (11, 16); the mode of the ergometer should be isokinetic, as it allows for maximal power generation, while the cadence is generally advised to be 130 or more revolutions per minute (17). Regarding the duration Langley et al. have suggested that 10 seconds is the optimal number (16), but their suggestion has been scrutinized (18).

If the chosen modality is running, the treadmill ergometer is typically selected as a device, but research has also been done on field tests. Test duration should again be in the range of 10 to 14 seconds, but research is far less than cycling, so a concrete recommendation is not possible. One study showed best results with a test duration of 12 seconds (19). Preferably a non-motorized treadmill should be used, as it can allow individual adjustment of the maximal effort with higher intensity (20).

Figure 2. Recommended Protocol for Cycling VLamax testing.

Figure 3. Recommended Protocol for Treadmill VLamax Testing.

Although not as common, VLamax testing can also be done for swimmers, rowers, kayakers, and non-specifically, using isokinetic dynamometers (2). Any of these modalities can be used depending on the athlete's specific discipline, although for simplicity very often these athletes undergo standard cycling ergometer testing. Swimming, for example, presents specific challenges such as the sampling process of blood lactate in a pool, where if the practitioner is not careful, the samples can get easily contaminated, but still overall it is practically feasible (21). Isokinetic strength tests and their use for VLamax are understudied with only a handful of studies in the literature exploring their use (22), thus this type of exercise remains a field to be explored, since it

can be a compromise in testing, combining various performance tests into one procedure.

Regarding the sample taking for blood lactate measurements, they are done before the test at rest once and after the test multiple times, to derive the point of maximal lactate concentration. Typically the number of samples is different, depending on how fast the lactate level drops. In most study protocols, the measurements are done every minute after the test, from capillary blood samples from a finger or earlobe. Different groups propose different concepts for the termination of sample taking. It has been described in the literature that sampling can stop after a decline is noted in 1 or more samples from the maximal recorded lactate level. Other groups determine the maximum concentration after they record a lactate value 0.5 or 1 mmol/l below the maximum recorded one (2, 11). The chosen protocol is up to the practitioner, it is of course better to be more rigorous for better accuracy and reproducibility of the results.

VLamax calculation

The formula that was derived by Mader et al. (1, 4) consists of the following:

$$VLamax = \frac{La_{max} - La_{rest}}{t_{exer} - t_{alac}}$$

In this equation four elements are crucial for a meaningful calculation. *'La_{rest}'* refers to the blood lactate level that was measured at rest just after a brief and easy warm-up. *'La_{max}'* is the maximal blood lactate level that was measured after the test. This value is divided by the *'t_{exer}'*, referring to the whole duration of the test, minus the *'t_{alac}'*, which is the time interval, where the main supplier of ATP is the phosphagen system (alactic time). From the described components, the 't_{alac}' is the one where the calculation needs to be precise, as it can change the result substantially. The alactic time can be derived via several ways from power measurements, or it can be a fixed number, different researchers apply it in their preferred way. As of now, studies suggest that the best reproducibility and reliability of the procedure is achieved if a fixed alactic time is used (11). The fixed number t_{alac} is derived from the simulation study of Heck et al. (4), which proposes that it varies with the duration of the exercise. For a standard test of a duration between 10 and 15 seconds the alactic time varies between 3 and 3.5 seconds.

After calculation VLamax varies between 0.1 and 0.8 mmol/L/min, which are considered average values. Higher values suggest high glycolytic capacity which can translate to better performance in explosive short-duration sports, whereas lower values are typically seen in endurance trained athletes, where the anaerobic glycolytic capacity is not that high but also not needed (2, 11).

Conclusion

VLamax is a novel in adoption, albeit not in origin, metric for the anaerobic glycolytic capacity of athletes. It is one of a small number of tests that directly measure anaerobic metabolism and as such has only the capacity to grow in importance for athletic performance analysis. Standardisation of the practical aspects is still lacking, but recently researchers have been acknowledging this problem and efforts are underway to correct this. Still the described recommendations are more or less accepted as norm and are sufficient for everyday practice. It remains to be seen how VLamax is going to affect performance analysis and results in the future.

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A rare branching pattern of the celiac trunk: a case report of quadrifurcation

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The celiac trunk is the first unpaired visceral branch of the abdominal aorta. In classical anatomy, it is described as a trifurcation consisting of the left gastric artery, the common hepatic artery, and the splenic artery. It supplies the distal third of the esophagus, the stomach, the proximal part of the duodenum, the liver, the gallbladder and biliary tree, the pancreas, the spleen, the greater and lesser omentum. Anatomical variations of the celiac trunk are not uncommon. The quadrifurcation of the celiac trunk is a rare variant branching pattern, comprising four branches: the three typical branches and one anomalous branch. During a routine dissection of a female cadaver fixed in formalin at the Department of Anatomy, Histology, and Embryology of the Medical University of Plovdiv a quadrifurcation was observed – the celiac trunk gave rise to the left gastric artery, the common hepatic artery, the splenic artery, and the dorsal pancreatic artery. The three typical branches followed their normal courses, whereas the additional artery ran inferiorly along the dorsal surface of the pancreas. The dorsal pancreatic artery typically originates from the splenic artery, if present, and commonly supplies the body and tail of the pancreas. Awareness of the anatomy and variants of the celiac trunk is crucial for radiologists and surgeons performing invasive diagnostic, therapeutic, and surgical interventions in the upper abdomen, including endovascular procedures, hepatobiliary surgery, liver transplantation, splenectomy, gastrectomy, and pancreaticoduodenectomy.

Keywords: *celiac trunk, quadrifurcation, dorsal pancreatic artery*

Introduction

The celiac trunk is the first unpaired visceral branch of the abdominal aorta. In classical anatomy, it is described as a trifurcation consisting of the left gastric artery, the common hepatic artery, and the splenic artery. Anatomical variations of the celiac trunk are not uncommon. The quadrifurcation of the celiac trunk is a rare variant branching pattern, comprising four branches: the three typical branches and one anomalous branch. The prevalence of quadrifurcation was 1,8%, according to Triantafyllou et al. [1]. The dorsal pancreatic artery (DPA) originated from the celiac trunk in 11,9% of the cases, according to Rousek et al. [2].

Case presentation

During a routine dissection of a female cadaver fixed in formalin at the Department of Anatomy, Histology, and Embryology of the Medical University of Plovdiv a quadrifurcation of the celiac trunk was observed. The celiac trunk gave rise to its three typical branches – left gastric artery, the common hepatic artery, the splenic artery, as well as an additional aberrant branch – the dorsal pancreatic artery (Fig. 1). The common hepatic artery (CHA) and the splenic artery (SA) had equal diameters of 6 mm. The smallest branch was the left gastric artery (LGA) – 3 mm in diameter. The DPA arose between the CHA and the SA and measured 3.5 mm in diameter and 2.2 cm in length. The three typical branches followed their normal courses. The DPA was located to the left of the portal vein. The dorsal pancreatic artery (DPA) descended posterior to the splenic vein and, at the upper border of the neck of the pancreas, divided like an inverted "T" into two terminal branches: right (diameter 2 mm) and left (diameter 2.5 mm). The right branch ran horizontally toward the head of the pancreas, posterior to the superior mesenteric vein. It gave off a descending branch and formed an anastomosis with the inferior pancreaticoduodenal artery, a branch of the superior mesenteric artery. The left branch ran horizontally toward the tail of the pancreas as the transverse pancreatic artery (Fig. 2). The DPA typically originates from the splenic artery, if present, and commonly supplies the body and tail of the pancreas. In our case, it arose from the celiac trunk (Fig. 3).

Figure 1. Quadrifurcation of the celiac trunk: CT – celiac trunk; LGA – left gastric artery; EB – esophageal branch; SA – splenic artery; DPA – dorsal pancreatic artery; CHA – common hepatic artery; PHA – proper hepatic artery; GDA – gastroduodenal artery; RHA – right hepatic artery; LHA – left hepatic artery; PV – portal vein; SV – splenic vein; S – stomach; P – pancreas; L – liver.

Figure 2. Dorsal pancreatic artery: DPA – dorsal pancreatic artery; RB – right branch; LB – left branch; SMA – superior mesenteric artery; IPDA – inferior pancreaticoduodenal artery; SV – splenic vein; SMV – superior mesenteric vein; P – pancreas.

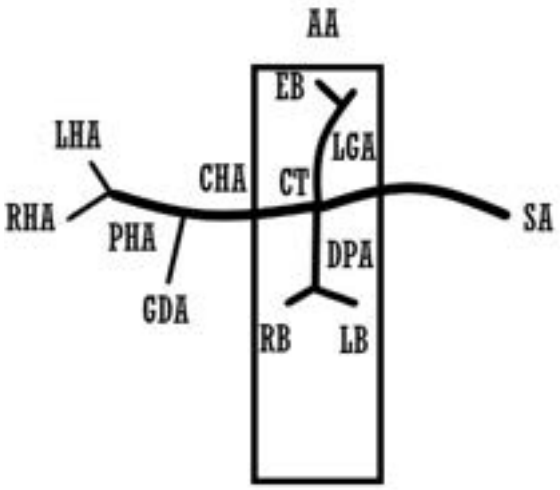

Figure 3. Quadrifurcation of celiac trunk (schematic illustration): AA – abdominal aorta; CT – celiactrunk; LGA – leftg astricartery; EB – esophageal branch; SA – splenic artery; DPA – dorsal pancreatic artery; RB – right branch; LB – left branch; CHA – common hepatic artery; PHA – proper hepatic artery; GDA – gastroduodenal artery; RHA – right hepatic artery; LHA – left hepatic artery.

Discussion

Depending on the number of its branches, the celiac trunk is classified as bifurcation, trifurcation, quadrifurcation, pentafurcation, and hexafurcation [3]. Quadrifurcation consists of four branches, three of which are typical – left gastric artery, the common hepatic artery, while the fourth branch is anomalous. According to Triantafyllou et al. the prevalence of this branching type is 1,8%. [1]. The left (LIPA) and right (RIPA) inferior phrenic arteries are the most reported aberrant branches in quadrifurcation, with the LIPA being the most frequent variant – 41,1% [4]. Vasuki et al. reported about four cadavers with quadrafurcation of celiac trunk. In three of the cases, they found accessory hepatic artery and one renal artery originating from the celiac trunk [5]. Gawali et al. described a replaced right hepatic artery with unusual course with the typical three branches [6]. In a cadaveric study, a quadrifurcation of the celiac trunk was found, consisting of the left gastric, splenic, left hepatic, and right hepatic arteries [7].

The dorsal pancreatic artery usually originates from the splenic artery and supplies the body and tail of the pancreas. The dorsal pancreatic artery (DPA) was reported to originate from the celiac trunk in 11,9% of cases [2]. Similarly, Yamane et al. observed an origin from the celiac trunk in 10% of the cases [8]. Rusu and Manta reported a heptafurcation of the celiac trunk that included the dorsal pancreatic artery, along with the three typical branches, the left and right inferior phrenic arteries, and a replaced right hepatic artery [9]. Hemalini et al. described a quadrifurcation involving the dorsal pancreatic artery [10].

Conclusion

Awareness of the anatomy and variants of the celiac trunk is crucial for radiologists and surgeons performing invasive diagnostic, therapeutic, and surgical interventions in the upper abdomen, including endovascular procedures, hepatobiliary surgery, liver transplantation, splenectomy, gastrectomy, and particularly pancreatic surgery, such as pancreaticoduodenectomy and pancreatic transplantation.

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Intake of added sugars in childhood and metabolic consequences: connection with insulin resistance and energy metabolism

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Introduction: In modern dietary patterns, an increased intake of added sugars during childhood has been observed, coinciding with a rising incidence of pediatric metabolic conditions, including insulin resistance, obesity, and early cardiometabolic risk. Accumulating experimental and clinical evidence indicates that excessive sugar consumption disrupts energy metabolism and glucose homeostasis. **Aim:** The aim of this review is to assess the scientific evidence regarding the role of added sugars in childhood nutrition and to analyze the pathophysiological mechanisms through which excessive intake contributes to the development of insulin resistance and related metabolic disorders. **Materials and methods:** A systematic review of peer-reviewed publications indexed in major databases – PubMed and Google scholar was performed, focusing on evidence related to carbohydrate metabolism, insulin signaling, fructose metabolism, and dietary patterns in childhood. **Results and discussion:** The reviewed data indicate that high intake of added sugars is associated with impaired postprandial glycemic regulation and increased insulin secretion in response to elevated glycemic levels. These findings demonstrate a clear association between childhood dietary habits, energy imbalance, and early metabolic dysfunction. **Conclusion:** This review highlights the importance of limiting added sugar intake and improving the quality of dietary carbohydrates as key strategies for the prevention of insulin resistance. A deeper understanding of the underlying metabolic mechanisms may support the development of effective preventive and therapeutic approaches aimed at improving long-term metabolic health in children.

Keywords: *added sugars, insulin resistance, energy metabolism, childhood nutrition*

Introduction

Carbohydrates are a major macronutrient in childhood nutrition and represent one of the leading sources of energy for the growing organism. Digestible carbohydrates provide a substantial proportion of daily energy intake in early childhood and are essential for normal growth and development, as well as for maintaining the energy metabolism and physical activity in children (Stephen et al., 2012). Sugars contribute to total energy intake. In the context of childhood nutrition, however, it is necessary to clearly distinguish between naturally occurring sugars, which are present in fruits, vegetables, and dairy products, and added sugars, which are incorporated into foods and beverages during industrial processing or their preparation (Vos et al., 2017; Buyken et al., 2018). Scientific evidence focused on pediatric populations indicates that although carbohydrates are physiologically necessary, excessive intake of added sugars does not provide additional nutritional value and is associated with adverse metabolic effects, particularly when it displaces nutrient-dense sources of energy (Rupérez et al., 2019; Calcaterra et al., 2023). Within the overall dietary pattern of children and adolescents, foods and drinks with a high content of added sugars constitute a considerable share. Sugar-sweetened and fruit-flavoured beverages have been identified as a major source of added sugars in pediatric and adolescent diets and are associated with positive energy balance and increased metabolic risk (Rupérez et al., 2019; Calcaterra et al., 2023). A high intake of added sugars during childhood has been associated with overweight, obesity, insulin resistance, and early manifestations of cardiometabolic disturbances (Vos et al., 2017; Rupérez et al., 2019).

Aim

The present review aims to examine the role of added sugars in childhood as regulators of energy metabolism and a risk factor for insulin resistance. Particular emphasis is placed on the mechanisms of glucose absorption and metabolism, the specific characteristics of carbohydrate intake during childhood, and the importance of carbohydrate quality for physical performance and metabolic function. This review summarizes current scientific evidence on the association between added sugar intake and early metabolic disturbances in children and adolescents.

Materials and methods

A narrative review of the scientific literature was conducted using the PubMed database. Priority was given to systematic reviews, meta-analyses, consensus statements, and large observational studies addressing carbohydrate metabolism, sugar intake, insulin resistance, and pediatric metabolic health. Reference selection was guided by relevance to childhood nutrition and metabolic outcomes.

Results and discussion

The scientific literature uses varied terminology to describe sugars and their derivatives, such as total sugars, free sugars, added sugars, intrinsic and extrinsic sugars, non-milk extrinsic sugars (NMES), and caloric sweeteners (Stephen et al., 2012; Buyken et al., 2018). This heterogeneity reflects differences in classification based on dietary source, processing, and relevance for nutritional assessment and health outcomes, rather than fundamental chemical distinctions (Buyken et al., 2018). In nutritional and public health settings, classifications distinguish between sugars naturally present in whole foods and those added during food processing or preparation, due to their differing consequences for dietary assessment and health risk (Vos et al., 2017; Buyken et al., 2018). In this review, “added sugars” follows the World Health Organization definition, referring to monosaccharides and disaccharides added to foods and beverages by manufacturers, cooks, or consumers, and excluding sugars naturally present in intact fruits, vegetables, and milk (World Health Organization, 2015).

Tissue-specific effects of insulin signaling on glucose metabolism. Insulin promotes glucose uptake and storage as glycogen in muscle and as triglycerides in adipose tissue, while inhibiting lipolysis and glycogen breakdown. In the liver, insulin suppresses gluconeogenesis and glycogenolysis and stimulates glycogen synthesis and glycolysis. In the brain, insulin signaling modulates feeding behavior and energy expenditure. Pathways stimulated by insulin are indicated in green, whereas inhibited pathways are shown in gray (Ashrafi, 2007).

From a cognitive perspective, carbohydrates play a key role by guaranteeing an adequate supply of glucose, the primary physiological fuel for the brain (Stephen et al., 2012; Simpson et al., 2007). During childhood, sufficient glucose availability is required to meet the elevated metabolic demands associated with brain growth, synaptogenesis, and functional maturation (Simpson et al., 2007; Mergenthaler et al., 2013). Beyond energy supply, evidence suggests that the quality and type of dietary sugars may influence cognitive outcomes. A recent systematic review and meta-analysis reported that higher intake of free and added sugars is associated with poorer cognitive performance, particularly in domains of memory and executive function (Gillespie et al., 2024). Although findings are heterogeneous and causality cannot be inferred, these data indicate a potential adverse association between excessive intake of added sugars and cognitive function (Gillespie et al., 2024).

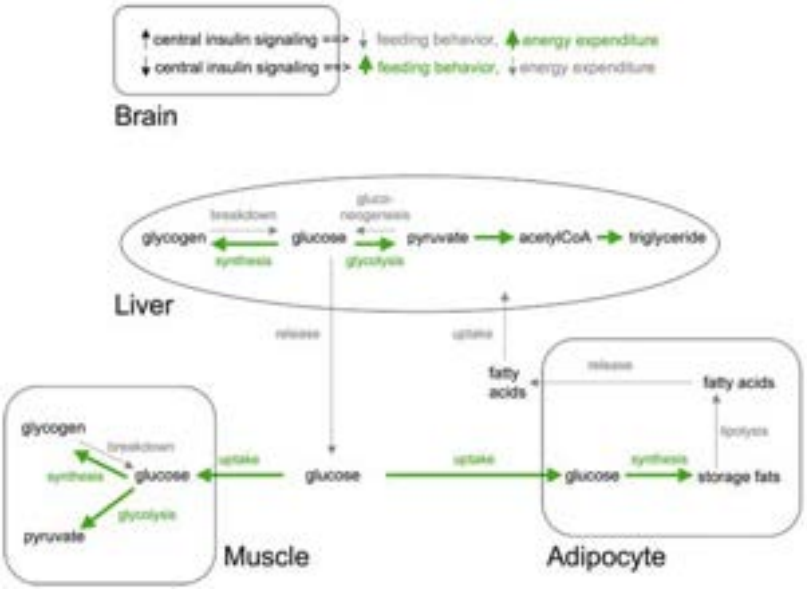

Figure 1. Systemic actions of insulin signaling in mammals (Ashrafi, 2007).

Across child populations, added sugars are consumed primarily via sugar-sweetened beverages and sweet, energy-dense processed foods. In recent NHANES-based analyses of U.S. youth, the leading sources among high consumers include sweetened beverages, sweet bakery products, candy, other desserts, and ready-to-eat cereals, with snacking occasions contributing substantially to added sugar exposure (Vinoy et al., 2023). Consistent with these patterns, pediatric-focused reviews and statements highlight sugar-sweetened beverages as a major contributor to added sugar intake and a key target for reduction strategies (Vos et al., 2017; Calcaterra et al., 2023).

Table 1. Total and added sugar values calculated using USDA Added Sugar Database, Food Pattern Equivalents Database (FPED), and NDSR database (adapted from Erickson & Slavin, 2015).

Food	Serving Size (g)	Total Sugars	Added Sugar – by Total Sugars (g)	Added Sugars by Available Carbohydrate (g)
Bread, white	25	1.3	1.0	1.0
Bread, whole wheat	28	2.9	1.4	1.9
Ice cream, vanilla	66	14.0	8.0	12.1
Cola, regular	368	33.0	33.0	35.2
Fruit punch	227	25.6	25.6	26.7
Candies, hard	3	1.9	1.9	2.8
Milk chocolate	43	22.1	20.2	20.2

Table 2. Recommended intake vs observed intake in children

	Recommended intake	Observed intake in children
Contribution to total daily energy	Reduction of free sugars to < 10% of total energy intake, with a conditional recommendation to further reduce to < 5% for additional health benefits (World Health Organization, 2015)	A substantial proportion of children exceed recommended limits; high consumers may obtain > 15% of total energy from added sugars (Park et al., 2023)

Pediatric nutrition studies indicate that parental dietary behaviors, household availability of sugar-rich foods and beverages, and feeding practices are central in affecting children's added sugar consumption (Vos et al., 2017; Rupérez et al., 2019). Higher parental intake of sugar-sweetened beverages and discretionary foods is consistently associated with increased added sugar intake in children. Early and continual exposure to sweet-tasting foods may reinforce preferences for sweetness and influence long-term dietary patterns (Rupérez et al., 2019; Calcaterra et al., 2023).

Expert consensus identifies postprandial glycemic response as an important physiological variable among pediatric populations, particularly with frequent consumption of high-sugar foods and beverages throughout the day (Vinoy et al., 2023). Repeated postprandial hyperglycemia increases β-cell secretory demand and may contribute to early dysregulation of glucose homeostasis (Luo et al., 2024). Mechanistic studies suggest that repeated glycemic excursions contribute to oxidative stress and inflammatory signaling, both of which are implicated in early metabolic impairment (Ceriello et al., 2008).

Recent pediatric-focused reviews describe hyperinsulinaemia as a common compensatory feature accompanying reduced insulin sensitivity in youth, particularly in obesogenic dietary contexts (Luo et al., 2024). In this setting, hyperinsulinaemia is presented as an intermediate phenotype that may precede clinically overt dysglycaemia and contribute to progressive metabolic deterioration (Luo et al., 2024).

Contemporary evidence describes insulin resistance as a result of combined disturbances in insulin signaling and substrate metabolism, driven by nutrient excess, intracellular lipid intermediates, inflammation, and altered tissue cross-talk (Petersen & Shulman, 2018; Chandrasekaran & Weiskirchen, 2024).

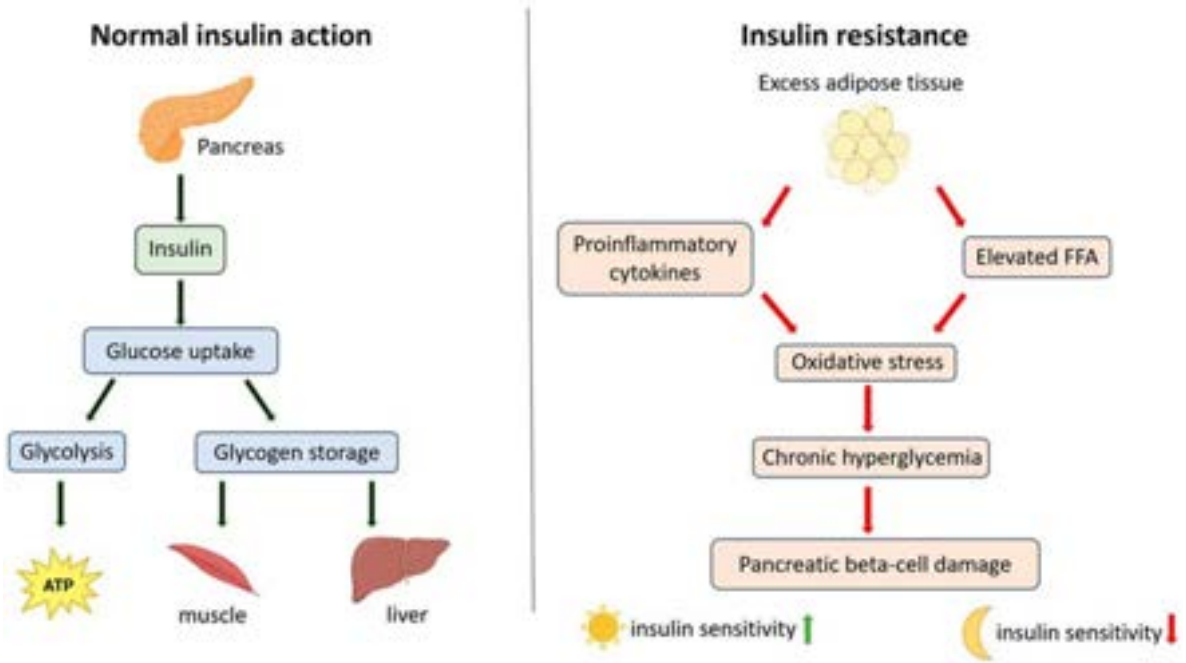

Figure 2. Mechanism of normal insulin action and insulin resistance. The diagram illustrates the differences between physiological insulin signaling and insulin resistance, highlighting the roles of pro-inflammatory cytokines, free fatty acids (FFAs), and oxidative stress (Nowacka et al., 2025).

Fructose metabolism exhibits distinct biochemical characteristics compared with glucose metabolism, particularly at the hepatic level. Under conditions of increased fructose availability, excess metabolites are preferentially directed toward de novo lipogenesis, leading to triglyceride synthesis and their incorporation into very-low-density lipoproteins (VLDL) for hepatic export. When the capacity for VLDL secretion is exceeded, triglycerides accumulate within hepatocytes, contributing to hepatic steatosis and providing a biochemical basis for the development of non-alcoholic fatty liver disease associated with excessive fructose intake (Bivolarska, 2025). The accumulation of hepatic triglycerides resulting from fructose-driven de novo lipogenesis is associated with the generation of bioactive lipid intermediates, including diacylglycerols and ceramides, which can impair insulin signaling pathways (Softic et al., 2020; Geidl-Flueck and Gerber, 2023). Recent evidence suggests that fructose-induced lipogenesis contributes to the development of hepatic insulin resistance, although this relationship is influenced by overall energy balance (Geidl-Flueck and Gerber, 2023).

Insulin resistance in childhood often develops subclinically and may be present long before abnormalities in fasting glucose become evident (Luo et al., 2024). Common early markers include elevated fasting insulin concentrations, increased homeostasis model assessment of insulin resistance (HOMA-IR), exaggerated postprandial insulin responses, and early lipid abnormalities, such as elevated triglycerides and reduced HDL cholesterol (Luo et al., 2024; González-Chávez et al., 2011). A strong association exists between excessive intake of added sugars and the development of overweight and obesity during childhood and adolescence (Vos et al., 2017; Calcaterra et al., 2023). Central adiposity is strongly linked to insulin resistance through mechanisms that include increased free fatty acid flux, adipose tissue inflammation, and altered adipokine secretion (Weihe & Weihrauch-Blüher, 2019).

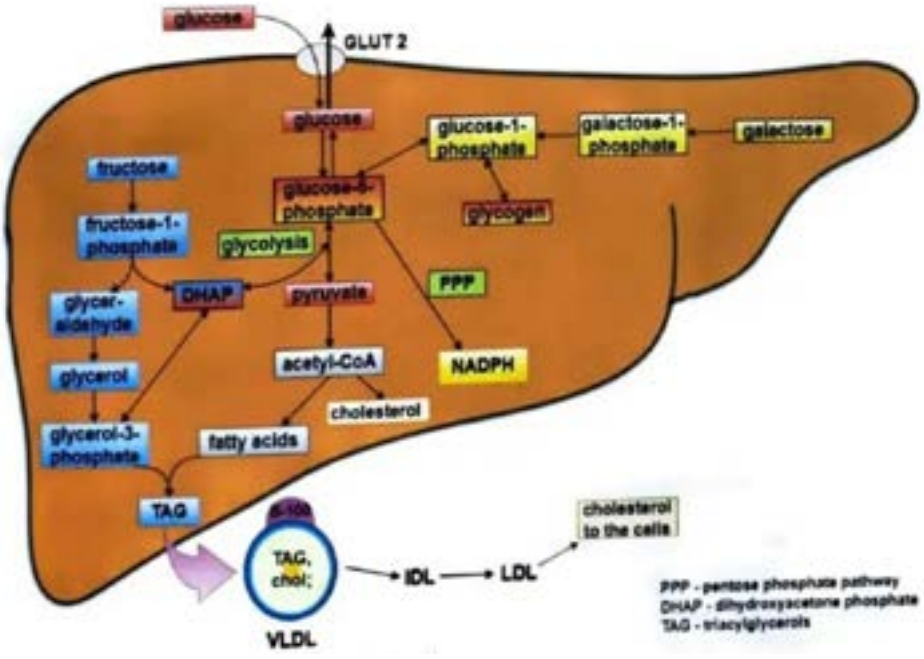

Figure 3. Carbohydrate metabolism in the liver (Bivolarska 2025)

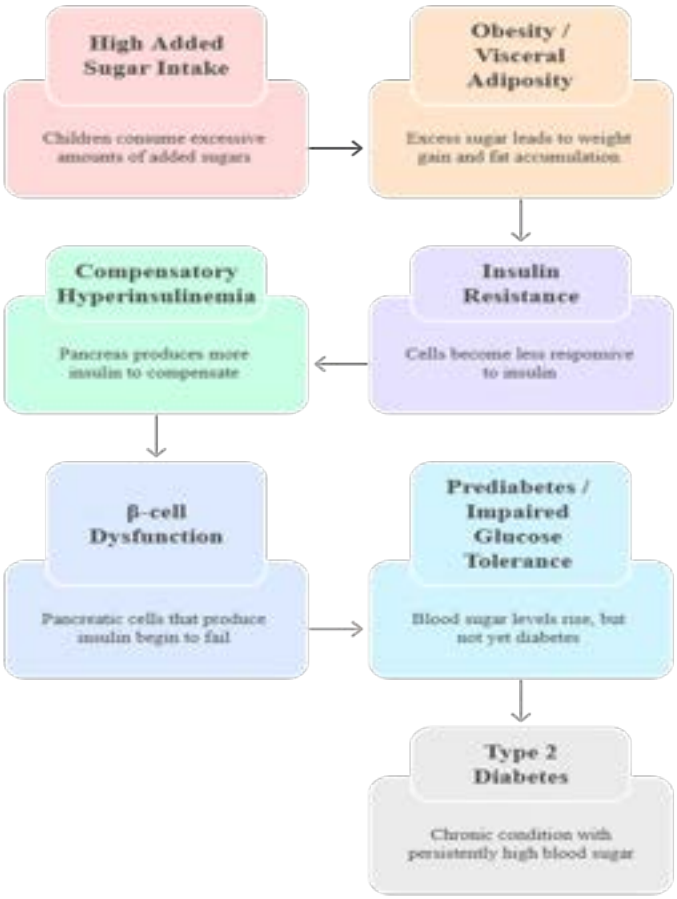

Figure 4. Childhood metabolic progression to type 2 diabetes

Longitudinal studies demonstrate that children with early insulin resistance or obesity are more likely to develop type 2 diabetes mellitus, non-alcoholic fatty liver disease, and cardiovascular disease in adulthood (Luo et al., 2024; Juonala et al., 2011). High intake of added sugars during childhood has been implicated in long-term cardiometabolic risk through sustained exposure to hyperinsulinemia, dyslipidemia, and hepatic fat accumulation, supporting the concept of early metabolic programming and the importance of preventive strategies during childhood (Calcaterra et al., 2023; Juonala et al., 2011). Diets high in whole grains, dietary fiber, fruits, and vegetables are associated with attenuated postprandial glycemic responses and improved insulin sensitivity relative to diets high in refined carbohydrates and added sugars (Buyken et al., 2018). Higher fiber intake slows carbohydrate absorption, reduces glycemic variability, and lowers insulin demand, therewith supporting metabolic stability during childhood and adolescence (Buyken et al., 2018; Reynolds et al., 2019). Systematic reviews demonstrate that school-based nutrition education programs are associated with improved dietary knowledge, reduced consumption of sugar-sweetened beverages, and healthier food choices among children and adolescents (Waters et al., 2011). Regulatory approaches targeting the food environment, including restrictions on marketing of sugar-rich foods and beverages to children, have been shown to reduce exposure to unhealthy products and impact purchasing and consumption patterns at the population level (Taillie et al., 2019). At the family and school level, practical strategies include limiting the availability of sugar-sweetened beverages, encouraging water and unsweetened dairy products, offering balanced meals rich in fiber and protein, and establishing regular meal patterns (Calcaterra et al., 2023; World Health Organization, 2015).

Conclusion

Excessive intake of added sugars during childhood is associated with adverse metabolic effects, such as postprandial glycemic dysregulation, compensatory hyperinsulinemia, and early development of insulin resistance. These changes may occur subclinically through critical growth periods and are linked to increased risk of obesity-associated metabolic and cardiovascular disorders later in life. Early prevention strategies that limit added sugars, improve carbohydrate quality, and promote healthy dietary patterns from an early age are essential. Upcoming research should prioritize longitudinal and interventional studies to clarify long-term outcomes and optimize preventive approaches among pediatric populations.

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Band-shaped insertion of pes anserinus: an anatomical variant with clinical significance in tendon graft harvesting

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Introduction: Pes anserinus (PA) is an important anatomical structure located on the anteromedial surface of the tibia, formed by the tendons of Sartorius, Gracilis, and Semitendinosus muscles. It exhibits considerable morphological variability with direct clinical relevance, particularly when the Gracilis and Semitendinosus tendons are used as autografts for anterior cruciate ligament reconstruction of the knee joint. **Aim:** The aim of the present study was to describe the morphology and insertion type of the tendons forming pes anserinus based on lower limb dissection and to evaluate its potential clinical significance during the surgical harvesting of tendon autografts. **Materials and methods:** The study was conducted through anatomical dissection of a lower limb fixed in a 10% formalin solution. The composition of the pes anserinus, the presence of accessory tendon bands, the type of insertion, and the relationship with the crural fascia were analyzed. **Results:** A classical pes anserinus was identified, composed of the tendons of the sartorius, gracilis, and semitendinosus muscles, inserting into the crural fascia on the anteromedial surface of the tibia. All tendons demonstrated a band-shaped type of insertion (width less than twice the width of the proximal tendon). **Conclusion:** The band-shaped insertion of all three components of the pes anserinus represents an anatomical variant of high clinical significance. Failure to recognize this variant may hinder the safe separation of the tendons during surgical harvesting of tendon grafts.
Keywords: *pes anserinus, tendon graft, band-shaped insertion, dissection*

Introduction

Pes anserinus (PA) is anatomically defined as the conjoined tendons of Sartorius, Gracilis, and Semitendinosus muscles inserting onto the anteromedial surface of the proximal tibia (1). It is named for its resemblance to a goose's foot (2). Structurally, PA comprises a superficial component formed by Sartorius (ST), Semitendinosus (STT) and Gracilis (GT) tendons which together create the so-called anserine plate through their fusion with the crural fascia. These tendons typically insert to the medial side of the tibial tuberosity (TT), contributing to both static and dynamic stabilization of the knee joint. Functionally, the muscles forming PA are involved in knee flexion and internal rotation and play an important role in rotational stability, particularly during dynamic activities. Beyond its biomechanical relevance, PA has significant clinical importance (3). GT and STT are widely used as autografts in anterior cruciate ligament reconstruction, as well as in procedures such as medial collateral ligament repair, patellar tendon augmentation, and medial knee reinforcement (4). Therefore, precise anatomical knowledge of this region is essential for safe surgical practice. Considerable morphological variability of PA has been described in the literature. Variations may involve the pattern of tendon convergence (convergent, divergent, or early convergence), the site and extent of insertion, and the presence of accessory tendinous bands arising from GT, STT, or, less frequently, ST. Accessory bands may also form adhesions with adjacent structures, including the tibial collateral ligament, Gastrocnemius aponeurosis, and surrounding fascial layers, reflecting differences established during embryological development (5). Cadaveric studies have classified PA morphology into multiple types and identified distinct insertion patterns, with reported variation frequencies differing across populations. Such diversity appears to be influenced by both ethnic and individual developmental factors (6). Failure to

recognize accessory tendons during graft harvesting may prolong operative time, complicate tendon isolation, reduce graft adequacy, or increase the risk of intraoperative complications such as premature tendon transection. Furthermore, unawareness of morphological diversity may lead to misinterpretation in radiological imaging and incorrect diagnosis of medial knee pathologies. Variations in the insertion type such as band-shaped configurations and their relationship with the crural fascia remain clinically underemphasized despite their potential impact on graft harvesting techniques.

Aim

Therefore, the present study aimed to describe in detail the morphology and insertion pattern of the tendons forming PA based on lower limb dissection and to evaluate their potential clinical significance during surgical harvesting of tendon autografts.

Materials and methods

The study was conducted through anatomical dissection of a lower limb fixed in a 10% formalin solution at the Department of Anatomy, Histology and Embryology in Medical University – Plovdiv. Upon dissection, the following morphological features of PA were evaluated: types of PA morphology such as number of bands creating PA; types of PA insertion (fan-shaped or bandshaped); the morphometric characteristics of tendon bands, such as their length and thickness (Table 1). A caliper was used for all measurements. Each measurement was carried out twice with an accuracy of up to 0.1 mm. The study procedure was approved by the Medical University – Plovdiv.

Results

A classical PA was identified, composed of the tendons of Sartorius, Gracilis, and Semitendinosus muscles, inserting into the crural fascia on the anteromedial surface of the tibia. All tendons demonstrated a band-shaped type of insertion (width less than twice the width of the proximal tendon) (Fig. 1). The proximal course of Sartorius, Semitendinosus, and Gracilis muscles was normal. Sartorius originated from the anterior superior iliac spine, Gracilis from the inferior ramus of pubic bone and Semitendinosus from the ischial tuberosity. The length of ST was 6 cm and the width of its insertion was 1.4 cm (Fig. 2). GT was attached to the medial part of the TT below ST, its length was 9.5 cm, while its insertion width was 0.9 cm (Fig. 3). STT was below the GT, its length 10 cm while its insertion width was 0.8 cm (Fig. 4). The common PA width is 3.1 cm.

Table 1. Morphometric measurements

	Sartorius	Gracilis	Semitendinosus
Insertion	Crural fascia	Medial part of the TT	Medial part of the TT
Tendon length	6 cm	9.5 cm	10 cm
Width of insertion	1.4 cm	0.9 cm	0.8 cm

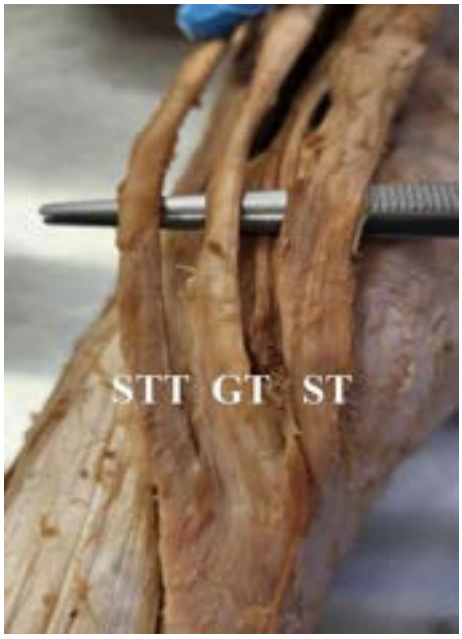

Figure 1. Band-shaped type of insertion of ST, GT and STT

Figure 2. Length of ST

Figure 3. Width of insertion of GT

Figure 4. Width of insertion of STT

Discussion

Numerous cadaveric and imaging-based studies of PA have documented substantial variability in tendon number, arrangement, insertion pattern, and fascial relationships (7). In the present study, a classical three-tendon configuration was observed; nevertheless, all components demonstrated a band-shaped insertion pattern into the crural fascia. This finding is clinically meaningful, as insertion morphology remains underemphasized compared with the more frequently discussed accessory bands. Olewnik et al. classified PA into six morphological types

based on tendon distribution and described three insertion patterns: short tendinous, band-shaped, and fan-shaped (8). The band-shaped type was defined as having a distal attachment less than twice the width of the proximal tendon. In our case, all three tendons fulfilled this criterion, forming a compact insertion into the crural fascia rather than a broad tibial attachment. While previous studies have often focused on accessory bands particularly those arising from the Semitendinosus, fewer investigations have addressed the surgical implications of insertion width and fascial integration. A band-shaped configuration may appear technically advantageous due to its narrower footprint; however, firm fascial fusion may complicate tendon separation during graft harvesting. The literature indicates that STT exhibits the highest frequency of accessory bands, whereas variations of GT are reported less consistently and ST variations are relatively rare (9). Some authors describe accessory structures in nearly all specimens examined, while others report much lower frequencies (10). Such discrepancies suggest that PA morphology is influenced not only by population-specific factors but also by individual developmental processes. From a surgical perspective, detailed knowledge of insertion morphology is essential. GT and STT are widely harvested for anterior cruciate ligament reconstruction and other ligamentous procedures. Accessory bands or atypical fascial attachments may increase operative time, complicate tendon stripper placement, and predispose to premature transection. In the present case, although no accessory tendons were identified, the firm band-shaped insertion into the crural fascia could require meticulous dissection to ensure safe graft retrieval (11). Failure to recognize such anatomical nuances may compromise graft length or integrity. The tendons of the Sartorius, Gracilis, and Semitendinosus function as dynamic stabilizers of the medial knee, contributing to flexion, internal rotation, and resistance to valgus stress. The present study has limitations, primarily its basis on a single dissected lower limb, which precludes generalization of prevalence data. Nevertheless, detailed morphometric assessment contributes valuable descriptive data and highlights the importance of insertion type as a clinically relevant parameter. Future investigations with larger cadaveric samples and correlation with imaging modalities such as ultrasound or magnetic resonance imaging would further clarify the in vivo relevance of these findings.

Conclusion

In conclusion, while the classical triple-tendon arrangement of PA was observed, the uniform band-shaped insertion of all three components into the crural fascia represents a notable anatomical variant. Recognition of such insertion patterns is crucial for optimizing surgical planning, minimizing intraoperative complications, and enhancing anatomical education.

Abbreviations

PA – Pes anserinus; ST – Sartorius tendon; GT – Gracilis tendon; STT – Semitendinosus tendon; TT – Tibial tuberosity

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Effects of vitamin D deficiency on mental health and fatigue

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Introduction: Vitamin D deficiency is a growing worldwide health concern that affects a considerable proportion of adults. It is well known that vitamin D plays a vital role in calcium regulation, bone mineralisation, and musculoskeletal health. In recent years, evidence has suggested that insufficient vitamin D levels may also be associated with adverse mental health outcomes, including depression, anxiety, cognitive impairment, and fatigue. **Aim:** The aim of this study was to generate evidence on the relationship between vitamin D deficiency and mental health outcomes in adults. **Materials and methods:** A narrative literature review was conducted using scientific sources such as PubMed, Scopus, Web of science and Google Scholar and other clinical guidance databases. The included studies were systematic reviews, randomised controlled trials, observational studies, and cross-sectional analyses examining serum vitamin D levels in relation to mental health, depression, and fatigue. **Results and discussion:** In observational studies, low serum vitamin D levels were linked to lower bone density, anxiety, cognitive dysfunction and fatigue. Vitamin D intake showed benefits for depression in individuals with deficiency, although outcomes varied depending on study design, baseline vitamin D status, and dosage. **Conclusion:** Vitamin D deficiency may negatively affect both skeletal and mental health and may contribute to depression and fatigue. Vitamin D intake appears most effective in individuals with confirmed deficiency. Further research is needed to clarify its broader clinical benefits.

Keywords: *vitamin D deficiency, mental health, depression, fatigue, supplements*

Introduction

Vitamin D deficiency (VDD) is a widespread global health issue, affecting approximately 30–50% of adults worldwide due to factors such as limited sun exposure, darker skin pigmentation, aging, obesity, chronic illness, and inadequate dietary intake.

While VDD is classically associated with bone and musculoskeletal problems, growing evidence suggests it may also contribute to mental health conditions including depression, anxiety, cognitive impairment, and fatigue.

Vitamin D receptors and the enzymes that process it are found in brain regions involved in mood regulation, cognition, and behaviour, providing a biological basis for this link.

Aim

The aim of this review is to synthesize current clinical evidence examining the relationship between vitamin D deficiency and mental health disorders in adults

Methodology

A narrative literature review was conducted using scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. Included studies comprised systematic reviews, randomised controlled trials and cross-sectional analyses examining the relationship between serum vitamin D levels and mental health outcomes such as depression and fatigue.

Results

Global prevalence of vitamin D deficiency

Vitamin D is a fat soluble secosteroid that plays an essential role in keeping calcium and phosphorus levels balanced, maintaining bone turnover, and supporting musculoskeletal health (Holick, 2007). On top of these well-known functions, vitamin D also affects the immune system, the way nerve cells communicate, brain protection, and the hypothalamic–pituitary–adrenal (HPA) axis. Through these pathways, it can have effects

on mood, thinking, and energy levels (Garcion et al., 2002). The standard way to measure vitamin D status is by checking serum 25-hydroxyvitamin D [25(OH)D]. Deficiency is defined as a level under 20 ng/mL, and insufficiency as 20–30 ng/mL (Boucher, 2022). The body makes vitamin D in the skin when exposed to ultraviolet B radiation, and smaller amounts come from the diet, such as oily fish, fortified foods, and supplements (Boucher, 2022; Holick, 2003). Vitamin D deficiency (VDD) is most often seen in people with darker skin, limited sun exposure, older age, obesity, and chronic diseases (Palacios et al., 2014). Major public health bodies like NICE and the NIH recognise VDD as a frequent and often underdiagnosed condition (Boucher, 2022; National Institutes of Health Office of Dietary Supplements, 2023; Holick, 2003). Vitamin D contributes to mitochondrial oxidative phosphorylation and cellular energy metabolism. Activation of the vitamin D receptor (VDR) increases transcription of genes involved in mitochondrial electron transport chain complexes and tricarboxylic acid (TCA) cycle activity, promoting efficient ATP synthesis (Eyles et al., 2013; Garcion et al., 2002). Deficiency may therefore reduce cellular energy production and contribute to fatigue.

Mental health and fatigue as public health concerns

Depression and anxiety account for a large share of illness, disability, and healthcare visits worldwide (Anglin et al., 2013; Parker & Brotchie, 2011; World Health Organization, 2017). Fatigue is also very common; it interferes with daily activities and lowers a person's ability to function (Nowak et al., 2016; Knutsen et al., 2014). Researchers have increasingly linked nutritional deficiencies particularly low vitamin D levels to both mental health issues and fatigue (Eyles et al., 2013; Grudet et al., 2014; Littlejohns et al., 2014; Goodwill & Szoeke, 2017).

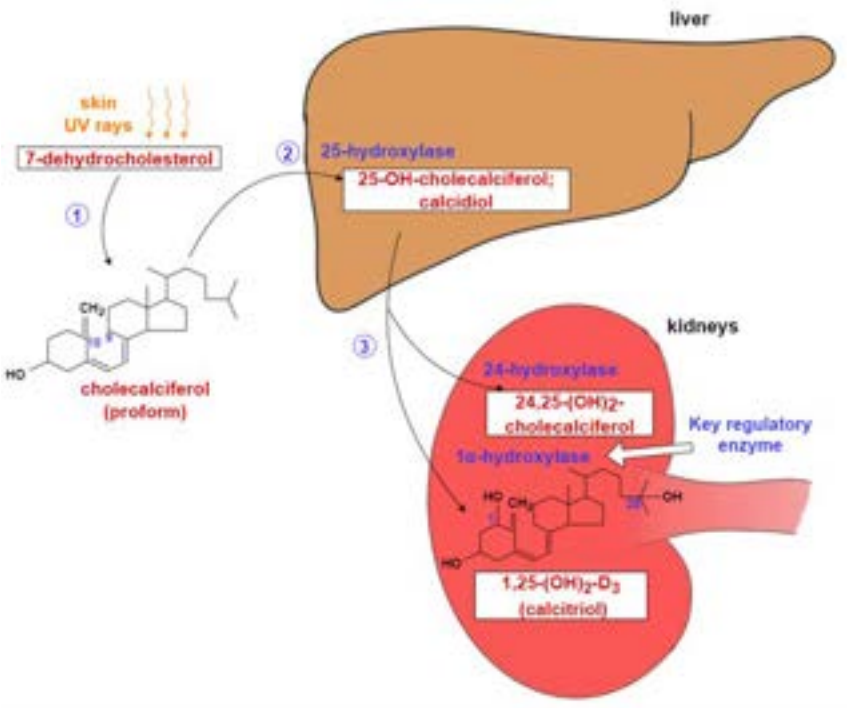

Figure 1. Synthesis of Vitamin D (Bivolarska, 2015)

Vitamin D and depression

Multiple observational studies consistently demonstrate that lower serum 25(OH)D concentrations are associated with increased depressive symptoms and a greater prevalence of depressive disorders (Anglin et al., 2013). Meta-analyses of randomised controlled trials suggest that vitamin D supplementation may provide modest improvement in depressive symptoms in individuals with confirmed deficiency, although findings remain heterogeneous because of differences in baseline vitamin D status, supplementation regimens, and study populations (Spedding, 2014; Gowda et al., 2015). Several biological mechanisms may explain this association. Vitamin D receptors (VDRs) and the enzyme 1α-hydroxylase are widely distributed in brain regions involved in mood regulation, including the hippocampus, prefrontal cortex, hypothalamus, and limbic system (Eyles et al., 2013). Vitamin D is believed to influence the synthesis and regulation of neurotransmitters such as serotonin, dopamine, and norepinephrine, all of which are strongly implicated in depressive disorders (Garcion et al., 2002). In addition, vitamin D appears to exert neuroprotective and anti-inflammatory effects. Deficiency has been associated with increased levels of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which may contribute to neuroinflammation and altered neuronal signaling observed in depression (Grudet et al., 2014). Vitamin D may also modulate hypothalamic–pituitary–adrenal (HPA) axis activity, potentially influencing the chronic stress response that is commonly dysregulated in depressive disorders.

Vitamin D and cognitive function

Low vitamin D levels have been associated with worse cognitive test scores, a higher risk of cognitive decline, and a greater chance of developing dementia (Eyles et al., 2013; Littlejohns et al., 2014; Goodwill & Szoeke, 2017). Overall, supplementation trials have not shown strong cognitive benefits, though meta-analyses suggest there may be small improvements in older adults (Littlejohns et al., 2014; Goodwill & Szoeke, 2017). Potential mechanisms linking vitamin D deficiency with cognitive decline include increased neuroinflammation, oxidative stress, impaired neuronal calcium regulation, and reduced neuroprotective signaling within the hippocampus and cerebral cortex.

Vitamin D and fatigue

Vitamin D deficiency has been associated with increased fatigue in both the general population and in clinical groups such as cancer survivors and individuals with metabolic disorders (Nowak et al., 2016; Knutsen et al., 2014). Clinical studies suggest that vitamin D supplementation may reduce fatigue symptoms in individuals with confirmed deficiency. The relationship between vitamin D deficiency and fatigue may be explained by several physiological mechanisms. Vitamin D plays an important role in skeletal muscle function, mitochondrial oxidative metabolism, and cellular energy production. Deficiency may impair mitochondrial ATP synthesis, resulting in reduced muscular efficiency and increased physical exhaustion. Furthermore, vitamin D deficiency has been linked to chronic low-grade inflammation, with elevated inflammatory cytokines potentially contributing to central and peripheral fatigue (Eyles et al., 2013; Garcion et al., 2002; Nowak et al., 2016). Vitamin D also affects neuromuscular signaling and muscle protein synthesis. Deficiency may therefore contribute not only to subjective tiredness but also to muscle weakness, reduced exercise tolerance, and impaired physical performance.

Vitamin D receptors are expressed throughout the central nervous system, particularly in regions responsible for emotional regulation, cognition, and behavioural control (Eyles et al., 2013). After activation, vitamin D can regulate gene transcription involved in neuronal growth, synaptic plasticity, and neuroprotection. One important mechanism involves modulation of neurotransmitter systems. Vitamin D contributes to the regulation of serotonin, dopamine, and norepinephrine synthesis, which are essential for mood stability and emotional processing. Deficiency may therefore disrupt neurotransmitter balance and contribute to depressive and anxiety symptoms (Garcion et al., 2002; Eyles et al., 2013).

Table 1. Correlation between vitamin D status, depressive symptoms, and fatigue severity based on findings from Anglin et al. (2013) and Nowak et al. (2016).

Vitamin D Status	Depression (%)	Fatigue (/10)
Deficient: (< 20 ng/mL)	42	7.2
Insufficient (20–30 ng/mL)	28	5.5
Sufficient (> 30ng/mL)	18	3.8

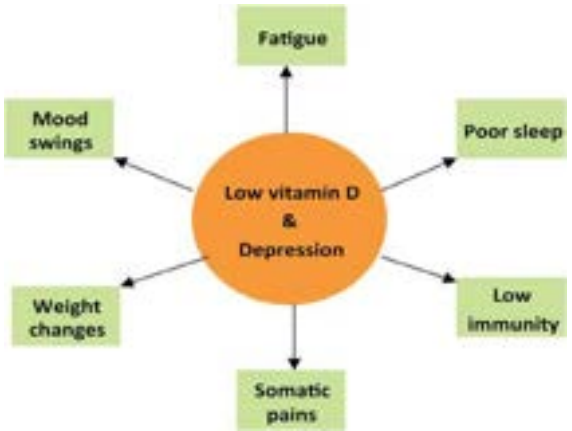

Figure 2. The effects of low vitamin D

Discussion

Vitamin D deficiency is increasingly recognised as a systemic condition with effects extending beyond bone and musculoskeletal health. Current evidence suggests that low serum concentrations may contribute to depressive symptoms, anxiety, cognitive impairment, and fatigue through multiple neurobiological and inflammatory pathways. Observational studies consistently demonstrate significant associations between vitamin D deficiency and adverse mental health outcomes; however, interventional studies investigating supplementation have produced variable results.

The inconsistency among supplementation trials may be explained by methodological heterogeneity, including differences in baseline vitamin D status, dosage regimens, treatment duration, participant characteristics, and outcome assessment tools. Importantly, several studies indicate that the greatest therapeutic benefit occurs in individuals with confirmed vitamin D deficiency, suggesting that correction of deficiency rather than indiscriminate supplementation may be clinically relevant.

Conclusion

Vitamin D deficiency is highly prevalent and has been implicated not only in musculoskeletal disorders, but also in adverse neuropsychiatric outcomes, including depression, cognitive dysfunction, and fatigue. Current evidence indicates that vitamin D supplementation may confer the greatest benefit in individuals with confirmed deficiency, particularly with respect to depressive symptoms and fatigue, although findings across studies remain heterogeneous. Consequently, correction of vitamin D deficiency may represent a cost-effective adjunctive intervention for improving both physical and mental health outcomes. Nevertheless, further large-scale randomized controlled trials are required to clarify causal relationships and establish optimal supplementation regimens.

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Circadian-metabolic axis effect on polyendocrine metabolic ovarian syndrome

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Introduction: Polyendocrine Metabolic Syndrome (PMOS) is characterised by hyperandrogenism, anovulation, ovarian cysts and menstrual irregularities, often presenting with acne, hirsutism, obesity, and alopecia. Individuals with PMOS have an increased risk of type 2 diabetes, insulin resistance, cardiovascular diseases, endometrial cancers, and obstructive sleep apnoea. An emerging component of PMOS pathophysiology involves disturbances in circadian rhythm regulation. **Objective:** The aim is to review the available literature on the roles of circadian rhythm disruption, metabolic dysregulation, and CLOCK gene alterations in the pathogenesis of PMOS. **Materials and methods:** A narrative literature review was conducted using databases, including PubMed and Science Direct, incorporating relevant human and animal studies. Circadian disruption alters cortisol and melatonin secretion patterns. Cortisol is simultaneously reliant on the circadian rhythm and disrupts the action of Melatonin. **Results/Discussion:** Patients present with elevated morning melatonin and evening cortisol levels. These alterations may enhance sympathetic nervous system activity, increase white adipose tissue, and promote obesity. Central and peripheral clocks affect the liver, adipose tissue, and ovarian granulosa cells through clock genes, including CLOCK, BMAL1, PER, and CRY. Dysregulation of these genes influences GLUT4 translocation, contributing to reduced sex hormone-binding globulin (SHBG) levels and increased Insulin-like growth factor-1 (IGF-1) levels, thereby worsening hyperandrogenism. Therefore, factors disrupting the circadian rhythm, such as jet lag and evening light exposure, may worsen PMOS. Melatonin supplementation has been shown to be beneficial in restoring circadian alignment and improving reproductive disturbances in PMOS. **Conclusion:** Circadian disruption is an important mechanism in the pathogenesis of PMOS, and its treatment is a promising therapeutic avenue.
Keywords: *Polycystic Ovarian Syndrome, Circadian rhythm, Clock genes, Melatonin, Metabolic*

Polyendocrine Metabolic Ovarian Syndrome (PMOS), formerly known as Polycystic Ovary Syndrome (PCOS), is no longer a purely reproductive disorder (1). Chronobiological disruption is emerging as a novel facet of PMOS pathophysiology, a pathological feedback loop that amplifies insulin resistance (IR) and hyperandrogenism, highlighting the mechanistic link between circadian biology and PCOS.

In PMOS, Hypothalamic-Pituitary-Ovarian Axis (HPO) dysregulation occurs due to rapid pulsatile frequency of GnRH (2, 5) to the pituitary gland. This results in a surge of LH than FSH, causing hyperandrogenism (2, 4). Insufficient FSH halts maturation, generating small, immature follicles, resulting in anovulation, infertility, and irregular periods (5). Hyperandrogenism (HA) is defined as an elevation in ovarian and adrenal androgen production with greater peripheral conversion of androgen precursors like testosterone, androstenedione, DHEA, etc (3, 4). The clinical presentation of HA presents with hirsutism, acne, alopecia, and menstrual cycle abnormalities, with chronic ovulation has serious metabolic and cardiovascular risk in the reproductive system (3, 4). Abnormal regulation of glucose metabolism and IR increases the risk of type 2 diabetes (5, 6). IR is a central pillar that drives PMOS because the lack of absorption of insulin by the cells results in bodily compensation by insulin overproduction resulting in HA and forms a vicious cycle of PMOS symptoms. To diagnose PMOS, a patient must have 2 out of the 3 requirements: HA, oligo- or anovulation, and polycystic ovaries, by ultrasound or raised levels of Anti-Müllerin hormone (2, 3, 5, 6, 7).

Research on PMOS pathogenesis is critical as it possesses a raised risk of diabetes mellitus, cardiovascular diseases, endometrial and breast cancers (5, 6).

Table 1. The four PMOS Phenotypes (3)

Phenotypical Groups	A	B	C	D
Oligomenorrhea	+	+		+
Clinical and/or biochemical hyperandrogenism	+	+	+	
Polycystic Ovaries	+		+	+

Circadian rhythm (CR) can be described as the 24-hour cycle of wakefulness and sleep controlled by light, environment, and various physiological processes (8). The CR depends on two major factors. Endogenous rhythmicity – the 24-hour period independent of light as well as the hormone melatonin and external factors: light, nutrition, and daily routine (9). Peripheral clocks (PC) with an innate endogenous rhythm are in tissues like the ovaries, liver, and adipose tissue. The suprachiasmatic nucleus (SCN), located in the hypothalamus, is the master clock responsible for maintaining the rhythm of the hypothalamic-pituitary adrenal (HPA) and HPO (10). It does so by synchronizing PC via its autonomic nervous system projections (secretion of cortisol, insulin/ glucagon, melatonin) and the HPA and HPO axes.

As shown in Fig. 1 on a molecular level, Clock genes rely on transcriptional and translation feedback loops in the peripheral tissue. BMAL1 and CLOCK (brain and muscle ARNT-like protein)1 promote transcription of Cry and period genes via their EBOX region. CRY and period molecules accumulate, dimerize, and repress the clock: BMAL1 loop – forming the core feedback loop. The secondary feedback loop with REV-Erb and ROR regulating an increase or decrease in BMAL1 expression.

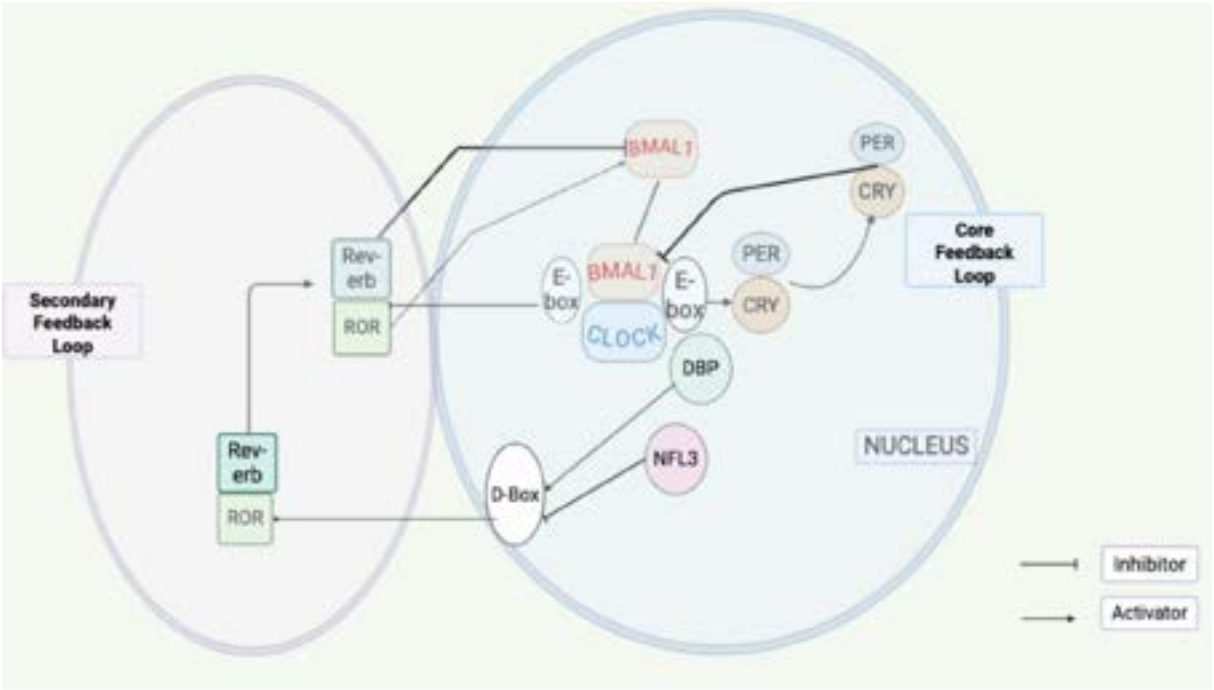

Figure 1. (Clock genes feedback loop) CREATED on biorender.com

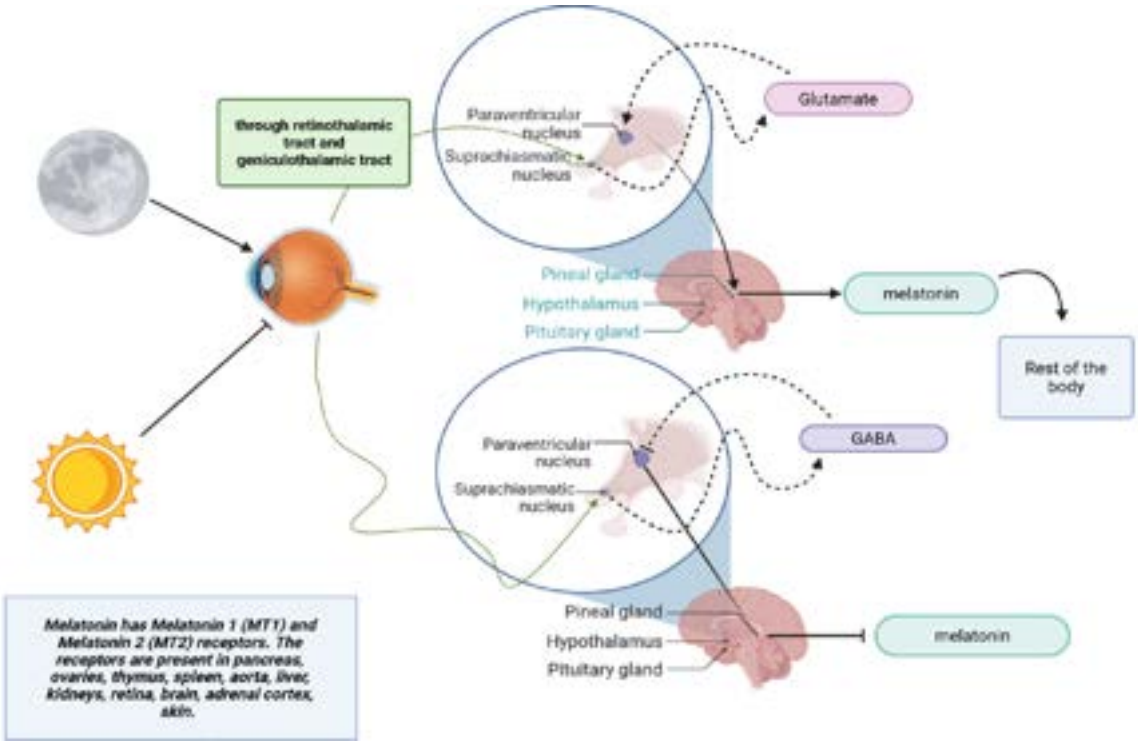

Figure 2. (Effects of light on melatonin secretion) *https://BioRender.com*

Melatonin (MT) is crucial in the hierarchy of clocks and assists the SCN in synchronization using light *(Fig. 2)*. During the day, melatonin is inhibited, causing increased cortisol. The light is detected by photosensitive retinal ganglion cells that express melanopsin and detected by the retina. That signal is transmitted to the SCN via the retinohypothalamic tract and synchronization of the Central clock (CC) occurs. SCN then releases gamma–aminobutyric acid (GABA) which inhibits synaptic transmission to the paraventricular nucleus (PVN). At night, MT is stimulated, causing a physiological decrease in cortisol subsequently followed by glutamate release from SCN to the PVN, which stimulates the pineal gland to begin melatonin secretion *(Fig. 2).*

MT influences ovarian function through its receptors, highlighting the importance of circadian biology for multidisciplinary approach involving gynecologists and endocrinologists in managing PMOS. MT exerts its effects through Melatonin 1 (MT1) and melatonin 2 (MT2) receptors expressed in the brain (SCN), testes, ovary, adrenal cortex, pituitary gland etc (11). PMOS patient presentation of oxidative stress, HA, and anovulation is a possible direct consequence of Chronobiological misalignment (3, 4). Studies showed a positive correlation between CR disruption (CRD) and increased LH pulsatility and expression in mice as well as increased LH and GnRH expression in human granulosa that was treated with MT supplementation with marked improvement (11, 12). Studies also showed an improvement in insulin, leptin, and visceral fat in older rats when treated with MT supplementation (11). A positive correlation was also found between decreased MT expression in follicular fluid and anovulation (symptoms of infertility and amenorrhea) (13)

MT acts as an anti-inflammatory agent and an antioxidant by being a scavenger for reactive oxygen species and reactive nitrogen species (1). Reduced melatonin could elevate chronic inflammation and increased oxidative stress commonly seen in PMOS.

Table 2. (Effects of melatonin on PMOS markers) (14)

PCOS Markers	The effects of Melatonin on PCOS markers
Hormones	↓ Androgens
Metabolic Health	↑ Insulin Sensitivity
Reproductive Health	↑ Induces Ovulation
Well-being	Improves mood disorders like depression and anxiety
Oxidative stress	↓
Cardiovascular risk	↓
Sleep-Wake cycle	Regulates sleep patterns and improves sleep quality

The CRD has many causative factors such as; artificial light exposure, shift work, jet lag, disorders (Parkinson's disease) and chronotype-related disorders. This disruption in turn affects; HPO, HPA and HPG axes which lose their normal timing, contributing to sleep, carbohydrates and lipid disturbances (impaired glucose tolerance, obesity, decreased night secretion of melatonin) via consequent internal clock misalignment and desynchronization (15).

Human CR can be classified into distinct phenotypes known as chronotypes identified as Morning (M), Neither (N), and Evening (E) and are found by identifying an individual's preferred time to be asleep or awake within a 24-hour period/cycle (16) Chronotypes are differentiated by melatonin profile, age, and peak activity. E types have peak activity in the afternoon. M types have earlier melatonin secretion and sleep and wake up earlier. N types also known as intermediate fit into either category and are often alternating between being a night owl or an early bird (16).Chronotype's contribution as an etiological factor in PMOS is rising. However, in middle-aged PMOS women, intermediate chronotype (IC) dominates rather than the proposed evening chronotype which is thought to be predominant among patients with PMOS. (17) A future suggestion is to examine correlations between chronotype and disease markers like cortisol levels, IR, and hirsutism which were omitted in the study.

In adolescent PMOS patients, there is evidence of a delayed offset of melatonin (DLMoff) in the morning. Normally, adolescents present with the late chronotype; however, this is more significant in PMOS women in the age category. Higher DLMoff is associated with higher free testosterone, and shorter sleep can increase HPA axis activity, thereby elevating cortisol levels and contributing to IR. In adolescents, early school start time is severely detrimental, as students attend classes during their biological night (18).

CRD by shift work has a profound effect on PMOS. In a cohort of 436 Chinese women, nightshift work nearly doubled the odds of developing PMOS (19). Supporting this, rats have the elevation of cortisol at 06:00 corresponding to evening cortisol levels in humans as rats are nocturnal animals – highlighting the disrupted stress–circadian axis *(20)*

Interplay between circadian and PMOS

The circadian clock directly affects the HPG axis in the hypothalamus and ovaries. The SCN exerts its effects on the female reproductive system via two main pathways, influencing LH and FSH levels and resulting in an imbalance. 1. The VIP-containing neurons fire directly to the GnRH, which results in the release of LH via the pituitary gland. 2. AVPV-kisspeptin neurons. In the AVPV nuclei, kisspeptin neurons stimulate the release of preovulatory GnRH/LH. Unlike VIP neurons, there is evidence that Kisspeptin

neurons have E2-Sensitive, E2-dependent / estrogen-sensitive circadian oscillator (21)

Within granulosa cells, clock gene oscillations directly regulate steroidogenesis. CYP19A1 is necessary for BMAL1–CLOCK-driven transcription and exhibits its own autonomous rhythm peaking at zeitgeber 16. (21) Reduced BMAL1 and upregulation of 5-alpha reductase genes shifts metabolism towards DHT, favoring more potent androgen formation. (22). Clock gene bidirectionally, regulate hormone production, but hormones like testosterone can disrupt clock gene expression via Steroidogenic Acute Regulatory protein, and therefore precipitate hormonal imbalance (23).

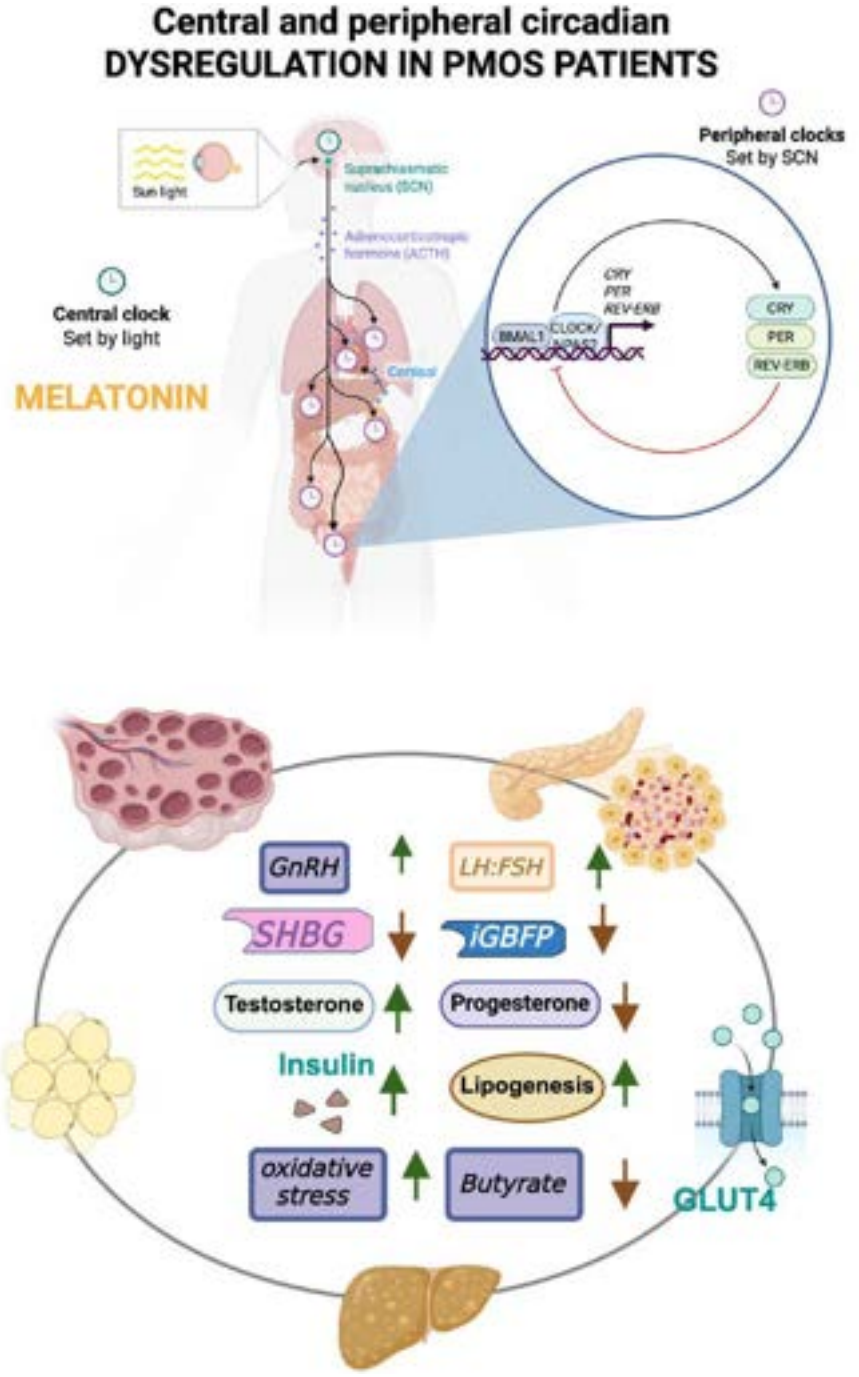

Figure 3. *Summary of Hormonal and Metabolic effects of CR (Central and Peripheral) dysregulation on PMOS Created in https://BioRender.com* Prolonged exposure to darkness can induce apoptosis in ovarian granulocytic cells, mediated by clock genes such as BMAL1, PER1, and PER2.

Hyperandrogenism and LH/FSH correlation with clock genes

PER and CRY correlate with MRNA expression of SHBG (sex-hormone binding globulin) and IGFB4 (insulin-growth factor binding protein). Decreased expression of these clock markers indicates elevated free testosterone. Per1 and Per2 dysregulation have been associated with the anovulatory symptoms of PMOS and are related to excessive LH. (24) PER2 has been associated with the induction of LH receptors and progesterone (via CYP17A1), which are expressed at higher levels in developing follicles (25). Dysfunction of critical gene regulators in the secondary clock feedback loop can affect mitochondrial biosynthesis. Evidence from PMOS rat models and KGN cells supports a significant reduction in REV-ERB expression. (26)

Insulin resistance (IR) and CLOCK

The BMAL1–CLOCK complex accumulates earlier in the zeitgeber cycle, while PER1–PER2 predominates later. In HEPG2 (human liver cell line) cell models, dysregulation of the zeitgeber organisation has been found to impair GLUT4 translocation in hepatocytes. Hyperinsulinemia can worsen PMOS by downregulating genes that increase apoptotic bodies, leading to follicular degeneration. (24, 25)

Butyrate, required for the mucosal lining, is often decreased in PMOS patients, possibly because IR affects mitochondrial melatonergic activity and there are speculations that influence the HPG axis resulting in the characteristic increased GnRH pulsatile activity. In fact, there is bidirectionality in its relationship as CRD hinders stimulation of butyrate in the gut bacteria (27).

Emerging circadian therapies for PMOS

MT supplementation has been shown to improve the serum LH/FSH ratio, IGF-1 levels, and SHBG levels, and thereby has positive effects in treating HA and hyperinsulinemia. Corrective light and dark therapy can improve glucose metabolism and decrease total androgen synthesis. (24) Alongside improving the traditional manifestations of PMOS, melatonin, by enhancing sleep quality, can improve depressive symptoms commonly encountered in these patients. However, side effects of melatonin supplementation must be considered like drowsiness and gastrointestinal disturbance and before clinical trials dosing and potential drug interactions with traditional PMOS treatments must be considered. (28) MT maintains PC integrity by blocking proteasomal degradation of BMAL1, which in turn suppresses pyroptosis in granulosa cells. (29) A double-blind placebo-controlled RCT study for 12 weeks showed potential decrease in hirsutism and testosterone but no alterations in the rhythm of menstrual cycles which needs to be controlled for future trials (30).

A more efficient method for improving CR and melatonin release is proper sleep hygiene. It is advisory for patients to avoid light, a main CR regulator (especially blue light) in the last couple of hours before sleep and have exposure to sunlight in the morning – if there is inadequate morning light, a light therapy device can be utilized. Likewise, blue-light filtering is recommended in the evening. Other circadian cues like meals and exercise should also be avoided in the last three hours before sleep. Sleep should be an appropriate length, and a specific regimen is necessary with similar times for bed and wake-up times.

SR9009, a REV-ERB agonist, can aid in restored mitochondrial biosynthesis and attenuated autophagy in KGN cells (immortalised human ovarian granulosa cell line). Western Blot and qRT-PCR techniques found reduced acetylation at specific histones which are normally responsible for suppressing autophagy. A combination of treatment with SR9009 and Nobiletin (ROR agonist) has shown promise in mice studies for enhancing glucose tolerance, the latter of which can stimulate insulin production (31).

Alternative medicine and emerging therapies

Quercetin, a flavonoid found in Chinese medicine, has been studied for its potential therapeutic applications in PMOS (32). It has a wide array of antioxidant properties that can be used to restore normal ovarian clock oscillations, with increased BMAL1 and reduced CRY/PER cycles. Quercetin, potentially combined with melatonin supplementation and light therapy, can provide the greatest benefit for addressing circadian disruption in individuals with PMOS. **Exosomes**, small cell-derived vesicles that shuttle RNAs and proteins between cells, have gained interest as therapeutic targets in PMOS due to their role in miRNA transport. IR and hyperandrogenism in PMOS may be due to dysregulation of miR-21 and miR-93. Several miRNAs are theorised to perturb PI3K signaling, a pathway regulated also by melatonin, resulting in excessive granulosa cells autophagy. Most notably, miRNAs can directly target core clock gene (CCG) expression, providing a molecular bridge between CRD and PMOS pathology. In the future, they can be emerging therapies although research is only beginning to uncover their intricate mechanisms. (33)

Conclusion

Circadian dysregulation may represent an important integrative framework for PMOS, linking insulin resistance, hyperandrogenism, and ovarian dysfunction through circadian disruption. Restoring circadian integrity may alter disease course and provide an adjunct therapeutic approach to PMOS management. Melatonin, light therapy and sleep hygiene are readily available approaches, while finer molecular mechanisms are being studied to provide further treatment options.

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Redefining heart failure treatment: from pathophysiology to phenotype-directed pharmacotherapy

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Introduction: Heart failure is a complex clinical syndrome characterized by structural, neurohormonal, metabolic, and inflammatory abnormalities that impair cardiac output and ventricular filling. Despite advances in therapy, it remains a major cause of morbidity and mortality worldwide. **Aim:** The aim of this review is to synthesize current understanding of heart failure pathophysiology and to evaluate established and emerging pharmacological therapies within a phenotype-directed treatment framework. **Methods:** A narrative review of current literature and major clinical trials was performed, focusing on guideline-directed therapies, recent pharmacological innovations, and mechanistic pathways underlying heart failure progression and heterogeneity. **Results and discussion:** Heart failure progression is driven by hemodynamic stress, neurohormonal activation, myocardial remodeling, and metabolic and inflammatory dysfunction. Established therapies, including inhibitors of the renin–angiotensin–aldosterone system, beta-blockers, mineralocorticoid receptor antagonists, and diuretics, improve outcomes by targeting these maladaptive pathways. Novel therapies such as sodium–glucose cotransporter 2 inhibitors, glucagon-like peptide-1 receptor agonists, non-steroidal mineralocorticoid receptor antagonists, and myotropes expand treatment options by addressing metabolic, fibrotic, and contractile abnormalities. A phenotype-based approach further differentiates therapeutic strategies across heart failure with reduced, mildly reduced, and preserved ejection fraction. **Conclusion:** Heart failure management is transitioning from a uniform therapeutic approach to a mechanism- and phenotype-directed strategy integrating traditional and emerging pharmacological agents, supporting more individualized and pathophysiology-based treatment.
Keywords: *heart failure; pharmacotherapy; neurohormonal activation; phenotype-directed therapy; cardiometabolic dysfunction*

Introduction

Heart failure (HF) is a complex clinical syndrome resulting from structural or functional cardiac abnormalities that impair ventricular filling or ejection (1). It affects over 60 million people worldwide and remains a leading cause of hospitalization, mortality, reduced quality of life, and healthcare expenditure (2).

Historically, HF management has focused on neurohormonal activation, particularly the renin–angiotensin–aldosterone system and the sympathetic nervous system (1, 3). Contemporary evidence, however, has expanded its pathophysiological framework to include metabolic, inflammatory, contractile, and molecular mechanisms, providing new opportunities for disease-modifying pharmacologic interventions (4).

Aim

This review aims to integrate established and emerging pharmacologic therapies in heart failure and to propose a phenotype-directed framework for mechanism-based treatment.

Methods

This narrative review synthesizes evidence from major randomized controlled trials, clinical guidelines, and peer-reviewed literature focusing on heart failure pathophysiology and pharmacotherapy.

Results and discussion

Pathophysiology of heart failure

Heart failure is defined as the inability of the heart to deliver sufficient oxygenated blood to meet metabolic demands or to do so only at the expense of elevated ventricular filling pressures (1).

1. Classification of heart failure

Heart failure is classified based on left ventricular ejection fraction (LVEF) and clinical presentation:

- **HFrEF (≤ 40%)**: systolic dysfunction
- **HFpEF (≥ 50%)**: diastolic dysfunction
- **HFmrEF (41–49%)**: intermediate phenotype
- **Right-sided HF**: systemic congestion (edema, ascites)
- **Acute decompensated HF (ADHF)**: acute worsening of symptoms

2. Core pathophysiologic mechanisms underlying the development of heart failure

A. Hemodynamic stress

At the hemodynamic level, reduced cardiac output leads to compensatory increases in preload, afterload, and heart rate. These mechanisms initially preserve perfusion but ultimately increase ventricular wall stress and accelerate disease progression (1, 5).

B. Neurohormonal activation: central driver of progression

Neurohormonal systems initially compensate for low cardiac output but later accelerate structural damage and worsen symptoms. Understanding these systems provides the mechanistic basis for modern HF pharmacotherapy.

These interconnected pathways, illustrated in **Figure 1**, create a self-perpetuating cycle of hemodynamic overload, maladaptive remodeling, and progressive cardiac dysfunction (3, 5). Additional contributors include metabolic and inflammatory dysregulation, which further impair myocardial efficiency and promote disease progression (4).

C. Myocardial Remodeling

At the structural level, myocardial injury and chronic stress result in maladaptive remodeling characterized by cardiomyocyte apoptosis, necrosis, and interstitial fibrosis (5). Remodeling patterns vary by phenotype, with eccentric remodeling in HFrEF and concentric remodeling in HFpEF (5).

D. Metabolic and inflammatory dysfunction

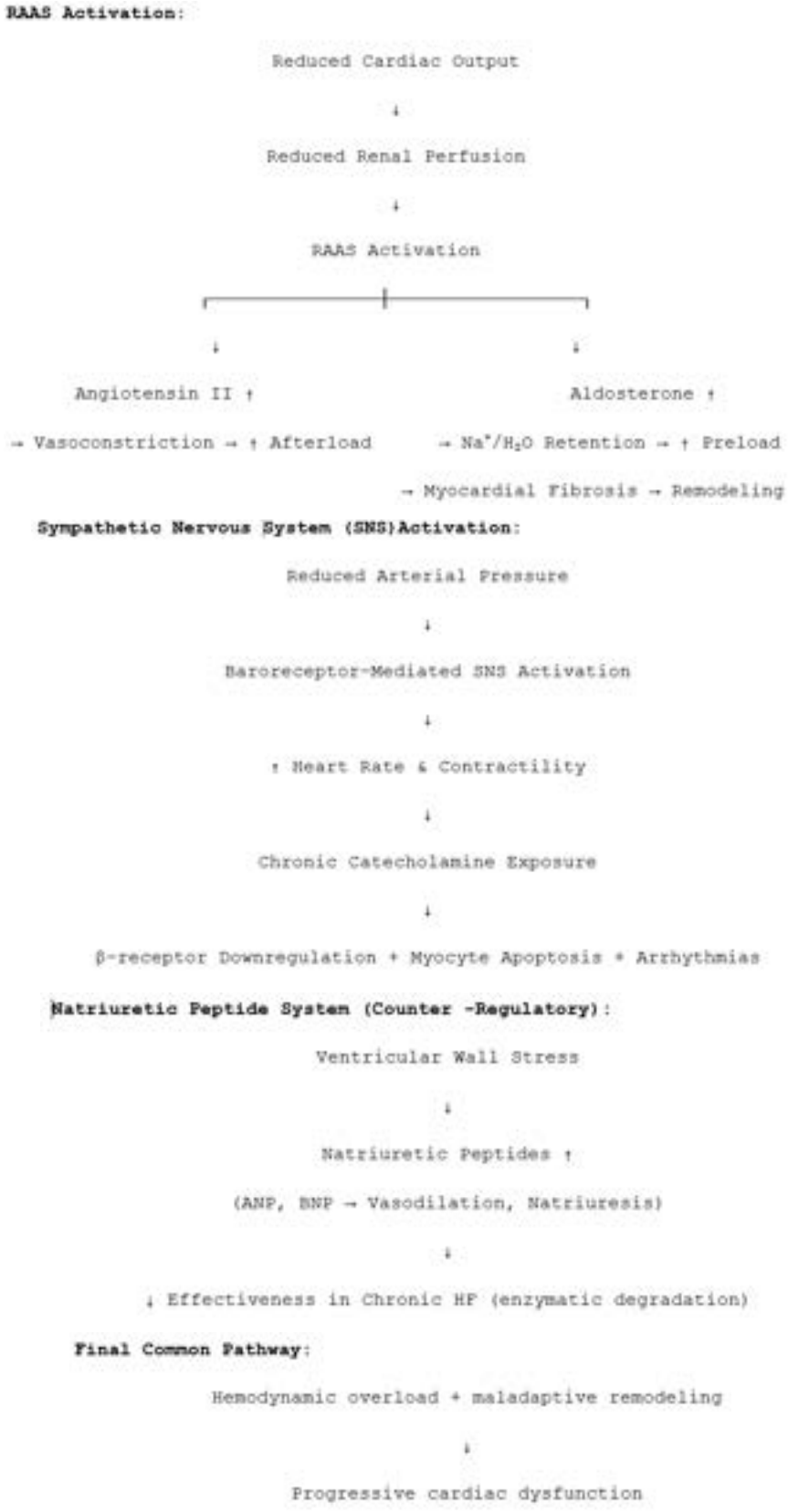
Beyond structural and neurohormonal changes, heart failure is also characterized by metabolic and inflammatory dysregulation, including insulin resistance, mitochondrial dysfunction, and activation of pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α, all of which contribute to impaired myocardial efficiency and disease progression **(4)**.

3. Classic pillars of pharmacotherapy: a mechanism-based framework

The following section outlines the foundational and emerging pharmacologic strategies in heart failure treatment, linking their mechanisms of action to the underlying pathophysiology.

Given the central role of neurohormonal activation, hemodynamic overload, and maladaptive remodeling, contemporary pharmacotherapy aims not only to relieve symptoms but also to interrupt disease progression (1, 3).

Each major drug class – RAAS inhibitors, β-blockers, mineralocorticoid receptor antagonists, and diuretics – targets specific maladaptive mechanisms.



Abbreviations: RAAS, renin–angiotensin–aldosterone system; SNS, sympathetic nervous system; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; HF, heart failure.

Figure 1. Neurohormonal and pathophysiologic mechanisms underlying heart failure progression.

Table 1. Mechanism-to-Therapy Mapping in Heart Failure: Linking Pathophysiologic Axes to Pharmacologic Targets

Pathophysiologic axis	Therapeutic targets
RAAS activation	ACEi/ARBs/ARNI, MRA
SNS activation	β-blockers
Sodium retention	Diuretics
Cardiac metabolism	SGLT2 inhibitors
Fibrosis	MRA, finerenone
Contractility	Myotropes
Inflammation	GLP-1 RA, biologics

Phenotype sensitivity

- HFrEF → neurohormonal dominant → full GDMT
- HFpEF → metabolic/inflammatory dominant → SGLT2i, GLP-1 RA
- HFmrEF → mixed response

4. Established guideline-directed therapy

Contemporary heart failure pharmacotherapy is anchored in several interdependent drug classes targeting hemodynamic load, neurohormonal activation, and myocardial remodeling.

Major pharmacologic classes in heart failure:

- RAAS inhibitors (ACE inhibitors, ARBs, ARNIs)
- β-blockers
- Mineralocorticoid receptor antagonists
- Diuretics
- SGLT2 inhibitors
- GLP-1 receptor agonists
- Novel agents (myotropes, RNA-based therapies)

4.1. RAAS inhibition: ACE inhibitors, ARBs, ARNIs

In heart failure, chronic activation of the renin–angiotensin–aldosterone system (RAAS) contributes to vasoconstriction, sodium retention, and adverse ventricular remodeling. ACE inhibitors reduce the conversion of angiotensin I to angiotensin II, thereby decreasing afterload, aldosterone secretion, and sympathetic activation. ARBs exert similar effects through selective blockade of the angiotensin II type 1 receptor (6).

Angiotensin receptor–neprilysin inhibitors (ARNIs), such as sacubitril/valsartan, combine RAAS inhibition with neprilysin blockade, enhancing endogenous natriuretic peptide activity. This dual mechanism promotes vasodilation, natriuresis, and antifibrotic effects. The PARADIGM-HF trial demonstrated significant reductions in cardiovascular mortality and heart failure hospitalization compared with ACE inhibitors, establishing ARNIs as first-line therapy in HFrEF (7).

Despite substantial benefits, residual cardiovascular risk persists, underscoring the need for additional therapeutic targets beyond neurohormonal modulation (1, 3).

4.2. β-blockers

Chronic sympathetic nervous system activation is a hallmark of heart failure progression, leading to tachycardia, increased myocardial oxygen demand, and cardiomyocyte injury. β-blockers (e.g., metoprolol succinate, bisoprolol, carvedilol) inhibit β1-adrenergic receptors, reducing heart rate, myocardial oxygen consumption, and renin release.

Long-term β-blockade promotes reverse ventricular remodeling, improves ejection fraction, and stabilizes cardiac electrophysiology. Major clinical trials such as MERIT-HF and CIBIS-II have demonstrated approximately a 30% reduction in mortality, along with decreased risk of sudden cardiac death (8).

4.3. Mineralocorticoid receptor antagonists

Aldosterone plays a key role in sodium retention, myocardial fibrosis, and vascular inflammation. Mineralocorticoid receptor

antagonists (MRAs), including spironolactone and eplerenone, block aldosterone signaling, thereby reducing fibrosis, improving ventricular compliance, and limiting adverse remodeling (9).

4.4. Diuretics

Diuretics play a central role in the symptomatic management of heart failure by reducing volume overload and relieving congestion. Loop diuretics (e.g., furosemide, bumetanide, torsemide) inhibit sodium reabsorption in the thick ascending limb of the loop of Henle, leading to potent natriuresis and diuresis.

These agents reduce ventricular filling pressures, improve pulmonary and systemic congestion, and enhance exercise tolerance. They are essential for stabilizing patients and enabling the initiation and optimization of disease-modifying therapies (1).

5. Emerging disease-modifying therapies

5.1. SGLT2 inhibitors

Sodium–glucose cotransporter 2 (SGLT2) inhibitors have emerged as a cornerstone therapy in heart failure, independent of diabetic status. By inhibiting renal glucose and sodium reabsorption, they induce osmotic diuresis and reduce intravascular volume without activating RAAS or the sympathetic nervous system.

Beyond hemodynamic effects, SGLT2 inhibitors improve myocardial energy efficiency, reduce inflammation, and provide renal protection. Landmark trials such as DAPA-HF and EMPEROR-Reduced demonstrated significant reductions in heart failure hospitalization and cardiovascular mortality across a broad range of patients (12, 13).

Although their clinical benefits are well established, the precise mechanisms underlying their cardioprotective effects remain incompletely understood and are likely multifactorial (12, 13).

5.2. Non-steroidal MRAs

Finerenone, a non-steroidal mineralocorticoid receptor antagonist, provides more selective receptor blockade with a reduced incidence of hyperkalemia and renal dysfunction compared with traditional MRAs. By attenuating myocardial and vascular fibrosis while preserving renal safety, finerenone broadens the therapeutic landscape, particularly for patients with HFpEF, in whom effective antifibrotic strategies have historically been limited (10, 18). Recent evidence from trials such as FINEARTS-HF suggests that finerenone reduces worsening heart failure events and cardiovascular mortality in patients with mildly reduced and preserved ejection fraction (10, 11, 18).

5.3. Myotropes

Beyond MRAs, novel mechanistic categories are redefining the scope of heart failure pharmacotherapy. Cardiac myotropes, such as omecamtiv mecarbil, enhance systolic performance by directly activating cardiac myosin, thereby improving contractility without increasing intracellular calcium or myocardial oxygen demand – offering a targeted alternative to traditional inotropic therapies (17, 19). Although clinical trials have demonstrated modest improvements in outcomes, their role in routine clinical practice remains under ongoing evaluation (17, 19).

5.4. Gene-directed therapies

Parallel advances in molecular medicine have introduced RNA-based and gene-directed therapies, including antisense oligonucleotides, small interfering RNA agents, and messenger RNA technologies, which enable targeted modulation of pathogenic protein expression implicated in cardiomyopathic and atherosclerotic processes (17, 22).

These approaches represent a promising step toward precision medicine, although they remain largely experimental.

6. Metabolic and inflammatory modulation in heart failure

6.1. GLP-1 receptor agonists

Glucagon-like peptide-1 (GLP-1) receptor agonists exert favorable cardiometabolic effects, including weight reduction, improved insulin sensitivity, and anti-inflammatory properties. These mechanisms are particularly relevant in heart failure with preserved ejection fraction (HFpEF), which is increasingly recognized as a heterogeneous syndrome driven by systemic metabolic dysregulation, chronic low-grade inflammation, endothelial dysfunction, and microvascular impairment (21, 23).

Evidence regarding the use of GLP-1 receptor agonists in heart failure remains limited to post hoc analyses and cardiovascular outcome trials. These data suggest neutral to modest effects on heart failure-related outcomes, particularly in patients with concomitant metabolic disease; however, dedicated randomized controlled trials specifically designed for heart failure populations have not yet been conducted (14–16).

Taken together, GLP-1 receptor agonists may represent a potential adjunctive therapeutic strategy in metabolically driven HFpEF phenotypes, particularly when used in combination with sodium–glucose cotransporter 2 inhibitors due to complementary cardiometabolic mechanisms. Their definitive role in heart failure management remains to be established in prospective, phenotype-specific clinical studies (14–16, 21).

6.2. Anti-inflammatory therapies

Additionally, inflammation has emerged as a central contributor to heart failure pathophysiology, particularly in HFpEF. Emerging anti-inflammatory biologics, such as interleukin-6 inhibitors like ziltivekimab, target the immunometabolic axis and have demonstrated reductions in inflammatory biomarkers and cardiovascular risk in early clinical studies (20, 21). These findings support the concept of inflammation-driven phenotypes and highlight new therapeutic targets beyond traditional neurohormonal pathways.

Despite their promise, these emerging therapies face important limitations, including limited long-term outcome data, high cost, and challenges related to accessibility and integration into guideline-directed medical therapy (19, 22).

7. Integration of established and emerging therapies

Modern heart failure management integrates traditional neurohormonal therapies – RAAS inhibitors, β-blockers, MRAs, and diuretics – with emerging pharmacologic strategies, including SGLT2 inhibitors, GLP-1 receptor agonists, non-steroidal MRAs, myotropes, and RNA-based therapies (1–10). This integrated approach allows phenotype-directed treatment, addressing congestion, structural remodeling, metabolic inefficiency, and systemic inflammation, and moving toward precision pharmacotherapy across the spectrum of heart failure.

8. Heart failure phenotypes and therapeutic matching

Heart failure is increasingly recognized as a heterogeneous syndrome comprising distinct clinical phenotypes characterized by different underlying pathophysiologic mechanisms and therapeutic responses (21, 23). This heterogeneity is particularly evident in heart failure with preserved ejection fraction, where systemic metabolic, inflammatory, and cardiorenal factors play a dominant role (21, 23). A phenotype-directed approach to pharmacologic therapy enables

more precise targeting of disease mechanisms and may improve clinical outcomes beyond traditional uniform treatment strategies (11, 21).

8.1. Heart failure with reduced ejection fraction (HFrEF)

HFrEF is primarily driven by neurohormonal activation and adverse ventricular remodeling, resulting in progressive systolic dysfunction (1, 3, 5).

Guideline-directed therapy – including ARNIs or ACE inhibitors/ARBs, β-blockers, MRAs, and SGLT2 inhibitors – targets these pathways and reduces mortality and hospitalization (1, 7, 8, 9, 12). This represents the most established model of mechanism-based treatment in heart failure (1, 3).

8.2. Heart failure with preserved ejection fraction (HFpEF)

HFpEF is a heterogeneous condition in which systemic factors such as metabolic dysfunction, inflammation, and vascular stiffness play a central role (21, 23). Unlike HFrEF, therapeutic strategies must be tailored to specific phenotypes.

a) Metabolic/obese HFpEF

This phenotype is characterized by obesity, insulin resistance, and low-grade inflammation, leading to impaired myocardial energetics (4, 21).

SGLT2 inhibitors improve cardiovascular outcomes and metabolic efficiency, while GLP-1 receptor agonists provide additional benefits through weight reduction and anti-inflammatory effects (12–16).

b) Hypertensive/aging HFpEF

This phenotype is associated with vascular stiffening, concentric remodeling, and myocardial fibrosis (5, 21).

MRAs and finerenone target fibrosis and inflammation, offering potential benefit in patients with preserved or mildly reduced ejection fraction (10, 11, 18).

c) CKD-associated HFpEF

In this phenotype, cardiorenal interactions, sodium retention, and volume overload are key drivers of disease progression (10, 21).

SGLT2 inhibitors improve both renal and cardiovascular outcomes, while finerenone contributes additional antifibrotic and anti-inflammatory effects (10–13, 18).

8.3 Heart failure with mildly reduced ejection fraction (HFmrEF)

HFmrEF represents an intermediate phenotype with overlapping features of HFrEF and HFpEF (1, 23).

Management is individualized, often incorporating therapies proven in HFrEF, particularly neurohormonal inhibitors and SGLT2 inhibitors (1, 11).

9. Limitations and challenges

Despite therapeutic advances, significant challenges remain. The heterogeneity of Heart Failure, particularly HFpEF, complicates treatment selection and limits the generalizability of clinical trial findings (21, 23).

Furthermore, many emerging therapies lack long-term outcome data, and economic barriers may restrict widespread implementation (19, 22). Addressing these limitations is essential for optimizing future care.

Table 2. Phenotype-directed pharmacologic approach in heart failure

Phenotype	Mechanism	Therapy
HFrEF	RAAS + SNS activation	ARNI + β-blocker + MRA + SGLT2i
HFpEF (metabolic)	Insulin resistance + inflammation	SGLT2i + GLP-1 RA
HFpEF (hypertensive)	Fibrosis + stiffness	MRA / finerenone
HFpEF (CKD)	Volume overload	SGLT2i + finerenone
HFmrEF	Mixed mechanisms	HFrEF-based therapy

10. Future perspectives

Future management will emphasize precision medicine, phenotype-specific therapies, pharmacogenomics, and digital health technologies (11–15). Combination strategies targeting complementary mechanisms (e.g., SGLT2 inhibitors + GLP-1 receptor agonists + finerenone) and risk stratification models may further optimize therapy selection, improve adherence, and enhance outcomes. These approaches aim to shift heart failure care from reactive to proactive, chronic, and individualized management.

Conclusion

Heart failure is a multifactorial syndrome driven by complex pathophysiologic mechanisms. While traditional therapies remain essential, emerging pharmacologic advances expand treatment possibilities. Integrating these approaches supports a transition toward personalized, mechanism-based care and improved long-term outcomes.

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III. Pharmacy and Pharmacotherapy Session

Psychobiotics: mechanisms of action and therapeutic potential – a review

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Introduction: Psychobiotics, defined as probiotics with neuroactive properties, are increasingly recognized for their ability to modulate mental health and cognitive function through the gut–brain axis. Their metabolites and signaling molecules interact with neural, immune, and endocrine pathways, offering potential therapeutic benefits in psychiatric and neurodegenerative disorders. **Aim:** This review aims to summarize current knowledge regarding the mechanisms of action of psychobiotics, their effects on cognitive and psychiatric disorders, and outcomes reported in recent clinical trials. **Materials and methods:** A literature search was conducted in PubMed and Google Scholar. Peer-reviewed articles, including systematic reviews and randomized clinical trial evidence published within the last decade, were analyzed. **Results and discussion:** Psychobiotics influence neurophysiology by modulating neurotransmitters such as serotonin and gamma-aminobutyric acid, altering the production of short-chain fatty acids, regulating inflammatory responses, and stabilizing the hypothalamic–pituitary–adrenal axis. Among the most frequently studied strains are those belonging to the genera *Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus, and Enterococcus.* Randomized clinical trials have reported improvements in mood, stress resilience, and certain cognitive parameters. Preclinical and early clinical studies in Alzheimer's and Parkinson's diseases indicate potential benefits, including reduced neuroinflammation and improved neuronal metabolism. However, outcomes vary depending on microbial strains, doses, intervention duration, and study design. Major limitations include methodological heterogeneity and the absence of standardized dosing protocols. **Conclusion:** Psychobiotics represent a promising adjunctive approach for cognitive enhancement and neuroprotection. Further well-controlled clinical trials are required to identify optimal strains, dosing strategies, and long-term efficacy.

Keywords: *psychobiotics, gut–brain axis, cognitive function, neurodegenerative diseases, psychiatric disorders*

Introduction

The gastrointestinal tract is home to a vast and complex ecosystem of trillions of microorganisms, which play a fundamental role in maintaining metabolic and physiological balance. Numerous studies have examined human microbiomes and their relationship to mental health [1].

Preclinical trials have shown that the gut microbiome interacts with both the enteric and central nervous systems through various physiological mechanisms, including neuroendocrine, immune, and neuronal pathways. Specifically, the role of gut microorganisms in neuroimmune modulation is critical, as disruptions in these processes are linked to the manifestation of psychiatric and neurological conditions such as depression, anxiety, autism spectrum disorders, and neurodegenerative diseases like Parkinson's and Alzheimer's. The composition of this microbial community is highly sensitive to environmental stressors associated with our modern lifestyle, such as poor diet,

stress, lack of physical activity, and overexposure to antibiotics. These factors can severely diminish microbial diversity and potentially lead to cognitive and emotional instability. Clinical trials with humans have shown that beneficial microorganisms can enhance cognitive functions and alleviate symptoms of anxiety, depression, and stress. This has led to the establishment of a new class of probiotics known as psychobiotics [2].

The term "psychobiotics" was first introduced to identify specific probiotics that, through the delivery of neuroactive substances, evoke measurable mental health improvements in individuals with psychiatric conditions. Unlike traditional probiotics, psychobiotics are specifically characterized by their neuroactive properties – their ability to produce or stimulate the production of neurotransmitters, neuromodulators, and anti-inflammatory molecules that directly impact the central nervous system (CNS) through the gut-brain axis. Examples of these molecules include gamma-aminobutyric acid (GABA), serotonin, dopamine, epinephrine, and short-chain fatty acids (SCFAs). They are essential neurotransmitters for normal brain function. Psychobiotics can also increase oxytocin levels, often called the "cuddle" hormone, and decrease cortisol levels, which is known as the "stress" hormone [3]. Some strains of psychobiotics, like *Lactobacillus plantarum* and *Lactobacillus brevis,* produce GABA – the main inhibitory mediator in the body, and serotonin, a hormone responsible for our good mood [4]. In addition, *Lactobacillus plantarum* and *Lactobacillus odontolyticus* can produce acetylcholine, a key neurotransmitter involved in memory and cognitive functions [5].

The gut-brain axis represents a complex communication system that links the CNS with the gastrointestinal tract. It is through this intricate pathway that psychobiotics exert their therapeutic effect. Compelling immunological evidence supports this communication, with observations indicating that the gut microbiota significantly influences the composition and function of glial and neuronal cells in the CNS. Furthermore, current research suggests that the integrity and permeability of the blood-brain barrier (BBB) are modulated by the presence of microbes [6]. By interacting with the neural, immune, and endocrine pathways of the gut-brain axis, these neuroactive organisms hold promising potential for the management of both psychiatric and neurodegenerative disorders.

Aim

This review aims to summarize current knowledge regarding the mechanisms of action of psychobiotics, their effects on neurodegenerative and psychiatric disorders, and outcomes reported in recent clinical trials.

Materials and methods

A literature review was undertaken using PubMed and Google Scholar databases. The following keywords and/or key phrases were used: "psychobiotics", "gut–brain axis", "neurodegenerative disorders", and "mechanism". The search yielded a significant number of articles, and manuscripts from the last five years were evaluated subsequently, with priority given to systematic reviews and clinical trials.

Results and discussion

1. Mechanism of action of psychobiotics

Although the exact pathways by which bacteria exert their neuroactive effects are not yet fully understood, current observations suggest a multi-targeted approach. They seem to interact with both the enteric

nervous system and the host's immune system, directly influencing how we handle stress and anxiety. From a physiological standpoint, this occurs through three key channels: first, by modulating the hypothalamic pituitary axis (HPA) and reducing systemic inflammation; second, by directly regulating host immunity; and third, by synthesizing various neurotransmitters, including proteins and SCFAs [7].

1.1. Neuroendocrine pathway – Hypothalamic pituitary axis (HPA)

The HPA axis acts as the body's main stress-management system. It consists of the hypothalamus, pituitary gland, and adrenal cortex, along with various hormones, secreted factors, and regulatory inputs. This axis triggers a hormonal cascade that begins in the hypothalamus and culminates in the release of cortisol from the adrenal cortex. While this response is crucial for maintaining homeostasis and mobilizing energy during stress, its chronic dysregulation underlies many psychiatric conditions. Persistent anxiety often results in hypercortisolemia, which is closely linked to cognitive decline and increased intestinal permeability [8, 9]. Clinical research shows that specific psychobiotic strains can significantly lower cortisol levels and reduce the pro-inflammatory cytokines associated with chronic stress. By restoring normal HPA axis function, phychobiotics help alleviate symptoms of anxiety and depression, while simultaneously enhancing cognitive function and emotional resilience [10, 11].

1.2. Immune pathway

Psychobiotics can exert their neuroactive effects by modulating systemic low-grade inflammation. This is a process tightly linked to psychiatric health. Pro-inflammatory cytokines can cross the BBB or stimulate prostaglandin production, thereby altering the concentrations of key neurotransmitters such as serotonin, dopamine, and glutamate [12–14].

A significant biochemical consequence of this inflammatory state is the diversion of tryptophan metabolism away from serotonin synthesis and toward the kynurenine pathway. This metabolic shift is driven by an enzyme, which is upregulated by cytokines that psychobiotics aim to suppress. By inhibiting this reroute, psychobiotics ensure higher serotonin levels and prevent the accumulation of neurotoxic kynurenine metabolites [15]. They can also reduce circulating pro-inflammatory markers and enhance anti-inflammatory cytokines, such as interleukin-10 (IL-10). For example, *Lactobacillus rhamnosus* GG has been shown in clinical studies to increase IL-10 levels in humans, helping to prevent neuroinflammation [16]. Furthermore, these microorganisms help preserve or restore the integrity of the BBB, shielding the CNS from inflammatory damage [17, 18].

1.3. Neural pathway

The microbiome can directly affect brain function by modulating the synthesis of neurochemicals, such as acetylcholine, serotonin, GABA, noradrenaline, dopamine, and SCFAs.

Serotonin is a key neurotransmitter that regulates mood, behavior, and essential biological processes in both the central and enteric nervous systems [19]. It is well established that the vast majority (90–95%) of the body’s serotonin is produced in the intestinal mucosa by enterochromaffin cells (ECs) and enteric neurons. Recent studies show that gut microbiota can enhance 5-HT biosynthesis from these ECs. This process is directly involved in gastrointestinal motility and platelet function [20, 21]. Specifically, strains like *Bifidobacterium longum* and

Lactobacillus rhamnosus have been found to reduce depressive-like behaviors and weight loss caused by chronic stress [22].

Dopamine (DA), norepinephrine (NE), and epinephrine are catecholamines, synthesized from the precursor amino acid tyrosine. These neurotransmitters are crucial for executive functions like learning, memory, motor control, and stress response. In the prefrontal cortex, NE and DA act as primary modulators of attention, decision-making, and inhibitory control. As a result, their dysregulation is a common sign of psychiatric conditions such as attention deficit hyperactivity disorder, post-traumatic stress disorder, and schizophrenia [23]. Certain psychobiotic strains have shown a significant capacity to influence these pathways. For example, studies with *Lacticaseibacillus rhamnosus* 12L and *Lactiplantibacillus plantarum* 8PA3 demonstrate that these microorganisms can directly produce DA and help metabolize it into NE and epinephrine [24]. These findings suggest that the intestinal microbiota serves not only as a source of neuroactive molecules but also as a key regulator of the body's catecholamine levels.

Gamma-aminobutyric acid is an essential neurotransmitter for regulating neurological processes, such as synaptic plasticity, neuronal excitability, and overall cognitive function. Recent studies of the human gut microbiota have identified specific strains, particularly *Lactobacillus brevis* and *Bifidobacterium dentium*, as highly effective GABA producers [4].

Short-chain fatty acids, mainly acetate, propionate, and butyrate, are produced by the anaerobic fermentation of indigestible fibers in the large intestine. These metabolites are vital for maintaining homeostasis, with functions ranging from stabilizing the intestinal barrier to regulating immune responses and fat metabolism [25]. Besides these effects, SCFAs act as essential mediators in the gut-brain axis. They contribute to neuroprotection by preserving the integrity of the BBB, modulating neurotransmission, and stimulating the synthesis of neurotrophic factors contributing to memory consolidation. Psychobiotic treatments like administration of *Bifidobacterium longum* 1714™ have been shown to alleviate stress- and anxiety-like behaviors, likely through pathways involving SCFAs [26, 27].

2. Human clinical trials

Recent double-blind, placebo-controlled trials provide concrete evidence that certain psychobiotic strains can significantly influence the human CNS. These studies use various diagnostic tools, ranging from psychological scales (like the Beck Depression Inventory (BDI)) to biochemical markers (cortisol and cytokine levels) and even functional neuroimaging (fMRI). Collectively, this evidence confirms that the gut-brain axis is not just a biological hypothesis, but a viable therapeutic target for treating many neurological and psychiatric conditions. The clinical data presented in Table 1 show the broad therapeutic potential of psychobiotics. These findings can be grouped into three primary categories:

1) Mental health and mood regulation: Studies [28] and [29] show a consistent reduction in depressive symptoms. Strains such as *B. longum* and *L. helveticus* have proven to effectively lower clinical scores on the BDI. This indicates that microbial intervention can serve as a powerful complementary treatment to traditional psychiatric therapy.

2) Cognitive function and neuroprotection: As shown in research [30], psychobiotic supplementation can lead to statistically significant improvement in the cognitive status of Alzheimer's patients. This suggests

that modulating the gut microbiome can affect neuroplasticity and lower oxidative stress in the brain.

3) Physiological stress response: Trials like [31] and [32] indicate that these beneficial bacteria can physically alter brain activity (observed via fMRI) and significantly reduce cortisol levels, the primary stress hormone.

However, clinical trial outcomes vary depending on microbial strains, doses, intervention duration, and study design. Major limitations include methodological heterogeneity and the absence of standardized dosing protocols.

Conclusion

Psychobiotics represent a promising adjunctive approach for cognitive enhancement and neuroprotection. Further well-controlled clinical trials are required to identify optimal strains, dosing strategies, and long-term efficacy.

Table 1. Review of clinical trials to study the effects of psychobiotics.

Type of study	Bacteria	Findings	Mechanism	Ref.
Randomized, placebo-controlled	*Bifidobacterium, Streptococcus, Lactobacillus*	Changed activity in emotion-processing regions via fMRI scans.	Direct gut-brain axis signaling.	31
Three-arm parallel RCT	*B. longum* R0175, *L. helveticus* R0052	Major depression: significant reduction in BDI scores.	Modulation of neurotransmitter synthesis.	29
Randomized, double-blind, placebo-controlled	*Lactobacillus plantarum* DR7	Stress and anxiety: marked decrease in cortisol and pro-inflammatory cytokines.	Reduction in plasma cortisol and IFN-γ.	32
Randomized, double-blind, placebo-controlled	*L. acidophilus, L. casei, B. bifidum, B. longum*	Alzheimer's: significant improvement in MMSE cognitive scores.	Reduction in oxidative stress	30
Randomized, double-blind, placebo-controlled	*Bifidobacterium longum* NCC3001	Reduced depression scores and amygdala reactivity to negative stimuli.	BDNF regulation and neuroactive compounds.	28

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Enhancing the oral delivery of curcumin via layer-by-layer assembly of chitosan and pectin on liposomal nanocarriers

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Introduction: Inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, represent chronic recurrent intestinal inflammation. Antioxidants are becoming increasingly important in the treatment of these diseases due to their ability to neutralize reactive oxygen species and limit oxidative damage to intestinal tissues. Curcumin, a polyphenol isolated from Curcuma spp., has strong antioxidant, anti-inflammatory, antitumor, and antibacterial properties. Its main problems are low bioavailability, stability and solubility, rapid metabolism and elimination, and the need for high doses to achieve an effect. The **aim** of the present work was to develop a smart oral delivery system for enhanced delivery of curcumin. **Materials and methods:** Curcumin-loaded, anionic liposomes were prepared by ethanol injection method. Subsequently, two consecutive layers of polysaccharides were deposited on the surface of the liposomes. All types of vesicles were evaluated in terms of their average size and zeta potential through light scattering techniques. Entrapment efficiency was evaluated by spectrophotometric analysis of the incorporated curcumin. The release profile of curcumin was tested via the dialysis bag method at two different medias, in order to test the ability of the formulation to withstand the gastric environment. **Results and discussion:** The formulated vesicles showed a relatively small increase in the average size and the polydispersity index remained low. Furthermore, the formulation showed a high resistance in acidic media and sustained release in pH 7.4. In conclusion, it can be said that the proposed formulation presents an opportunity for enhanced and controlled delivery of curcumin to the colon.

Keywords: *Layer-by-layer, liposomes, curcumin, oral delivery*

Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease and Ulcerative colitis, are chronic relapsing inflammatory disorders of the gastrointestinal tract that significantly affect patients' quality of life and impose a substantial burden worldwide [1, 2]. Although their exact etiology remains unclear, the pathogenesis of IBD is associated with an abnormal immune response, disruption of the intestinal barrier, alterations in the gut microbiota, and excessive production of reactive oxygen species (ROS). Increased oxidative stress contributes substantially to intestinal tissue damage and the perpetuation of inflammation [1, 2]. Consequently, compounds with antioxidant and anti-inflammatory properties have attracted considerable interest as potential therapeutic agents for the management of intestinal inflammatory conditions.

Among natural bioactive compounds, curcumin has emerged as one of the most extensively investigated phytochemicals [3–5]. Curcumin is the principal polyphenolic constituent of turmeric, obtained from plants of the genus Curcuma spp.. Numerous studies have demonstrated its broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antitumor, and immunomodulatory effects [4, 5]. Curcumin exerts its anti-inflammatory activity through modulation of several molecular pathways, including inhibition of nuclear factor kappa B (NF-κB), cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines [4, 6]. In addition, its potent free radical scavenging ability enables reduction of oxidative stress and protection of intestinal tissues from ROS-mediated damage. These properties make curcumin a promising candidate for the treatment of gastrointestinal

disorders, particularly inflammatory bowel diseases [4, 7].

Despite its considerable therapeutic potential, the clinical application of curcumin is severely limited by several unfavorable physicochemical and pharmacokinetic characteristics. Curcumin exhibits extremely low aqueous solubility and poor chemical stability, especially under physiological conditions [3, 8]. Furthermore, following oral administration, it undergoes rapid metabolism and systemic elimination, resulting in very low bioavailability. The hydrophobic nature of curcumin also restricts its dissolution in gastrointestinal fluids, thereby limiting intestinal absorption. Consequently, relatively high doses are often required to achieve therapeutic efficacy, which may reduce patient compliance and therapeutic efficiency. These limitations have stimulated intensive research toward the development of novel drug delivery systems capable of improving curcumin stability, solubility, and absorption after oral administration.

Nanocarrier-based systems have demonstrated significant potential for enhancing the delivery of poorly soluble compounds. Among them, liposomes are considered one of the most versatile and biocompatible delivery platforms [9, 10]. Liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core. Due to their unique structure, liposomes can encapsulate both hydrophilic and lipophilic substances, protecting them from degradation and improving their pharmacokinetic profile. Compared to other colloidal carriers, liposomes possess several important advantages, including biocompatibility, biodegradability, low toxicity, structural similarity to biological membranes, and the ability to enhance drug solubility and permeability [9–11]. Moreover, liposomal encapsulation can reduce premature degradation of encapsulated compounds within the gastrointestinal tract and provide sustained or targeted release profiles.

However, conventional liposomes may exhibit limited stability in the harsh gastrointestinal environment. Exposure to acidic gastric pH, digestive enzymes, bile salts, and mechanical stress can destabilize lipid vesicles and lead to premature drug leakage [12]. To overcome these limitations, surface modification strategies have been developed to improve liposomal stability and functionality [13, 14]. Among these approaches, polymer-coated or layer-by-layer liposomes have attracted increasing attention for oral drug delivery applications. The deposition of oppositely charged polymeric layers onto the liposomal surface can enhance vesicle stability, protect encapsulated compounds from gastric degradation, and modulate drug release behavior [13]. In addition, polymer coatings may improve mucoadhesion and prolong residence time in the gastrointestinal tract, thereby enhancing intestinal absorption and local drug accumulation [14, 15].

Natural polysaccharides are particularly attractive materials for liposome coating because of their biocompatibility, biodegradability, and low toxicity. Chitosan is a cationic polysaccharide obtained by deacetylation of chitin and is widely used in oral drug delivery systems due to its mucoadhesive properties and ability to transiently open tight junctions between epithelial cells, thereby enhancing paracellular transport [15, 16]. Chitosan coatings can also improve the physical stability of liposomes and protect them against acidic and enzymatic degradation [14, 16]. In contrast, pectin is an anionic polysaccharide known for its biocompatibility and pH-sensitive behavior. Pectin is particularly suitable for colon-targeted delivery because it remains relatively stable in the upper gastrointestinal tract but can undergo

degradation by colonic microflora [17, 18]. Furthermore, electrostatic interactions between positively charged chitosan and negatively charged pectin enable the formation of stable multilayer coatings through layer-by-layer deposition techniques. Such multilayer systems may provide enhanced protection of encapsulated compounds during gastric transit while allowing controlled release at intestinal or colonic pH conditions [13, 18].

Based on these considerations, the development of coated liposomal systems represents a promising strategy for improving the oral delivery of curcumin. The combination of liposomal encapsulation with polysaccharide multilayer coating may simultaneously enhance curcumin solubility, protect it from degradation in the gastrointestinal tract, prolong drug release, and increase its local availability in the colon. Therefore, the aim of the present study was to develop and characterize layer-by-layer coated liposomes for oral delivery of curcumin and to evaluate their physicochemical properties, encapsulation efficiency, and release behavior under simulated gastrointestinal conditions.

Results and discussion

Preparation of curcumin-loaded liposomes

In order to determine the optimal conditions for the preparation of curcumin-loaded liposomes, three formulations with varying lipid concentrations and lipid phase-to-curcumin ratios were developed, while maintaining a constant phosphatidylcholine (PC) to dipalmitoylphosphatidylglycerol (DPPG) ratio of 5:1. (Table 1).

The liposomes were prepared using the ethanol injection method. Phosphatidylcholine, phosphatidylglycerol, and curcumin were dissolved in 10 mL of absolute ethanol (99%). Subsequently, 50 mL of ultrapure water was heated to 65°C and the ethanolic lipid solution was injected into the aqueous phase under continuous stirring. The obtained suspension was stirred continuously for 1 h and then subjected to vacuum evaporation until complete ethanol removal. Finally, the suspension was stored under refrigerated conditions to allow complete vesicle formation.

The ethanol injection method proved suitable for the preparation of nanosized liposomes containing curcumin. The method is relatively simple, reproducible, and avoids the use of aggressive processing conditions that could compromise the stability of curcumin. Furthermore, the spontaneous formation of vesicles upon contact between the organic and aqueous phases allows efficient incorporation of lipophilic compounds within the lipid bilayer.

Characterization of liposomes

The prepared liposomes were characterized in terms of mean particle size, ζ-potential, and polydispersity index (PDI) using dynamic light scattering techniques (Table 2). In addition, the entrapment efficiency of curcumin was evaluated following ultracentrifugation of the liposomal suspension and spectrophotometric determination of the incorporated compound.

Table 1. Composition of model liposomes

Model	Lipid concentration (mg/mL)	Lipid phase : Curcumin
M1	1	5:1
M2	5	25:1
M3	10	50:1

Table 2. Characterization of liposomes – mean size, ζ – potential, polydispersity index

Model	Mean size ± SD (nm)	ζ-potential ± SD (mV)	Polydispersity index ± SD
M1	223.7 ± 2.7	-26.5 ± 0.15	0.53 ± 0.05
M2	167.9 ± 3.1	-52.08 ± 0.34	0.60 ± 0.07
M3	142.59 ± 2.1	-59.2 ± 0.37	0.99 ± 0.10

The obtained results demonstrated that increasing the lipid concentration led to a reduction in vesicle size and a more negative ζ-potential. The stronger negative surface charge observed for formulations M2 and M3 may be attributed to the higher proportion and improved distribution of the anionic phospholipid DPPG within the bilayer structure. Similar observations have been reported previously for anionic liposomal systems, where increasing lipid content and the presence of negatively charged phospholipids contributed to enhanced colloidal stability and more negative surface charge values [19, 20].

Among the tested formulations, M2 was considered optimal because it combined relatively small particle size with acceptable homogeneity and high curcumin incorporation. Although M3 exhibited the smallest vesicle size, its very high polydispersity index indicated a highly heterogeneous population and possible aggregation or instability of the system. In contrast, M2 showed a better balance between nanoscale dimensions, colloidal stability, and particle distribution, making it more suitable for subsequent coating procedures.

Coating of the optimized liposomal formulation

Following liposome characterization, formulation M2 was selected for further coating with chitosan and pectin layers. Five coating formulations with varying polysaccharide concentrations were prepared (Table 3).

Table 3. Preparation of coated liposomal models

Model	Chitosan (mg/mL)	Pectin (mg/mL)
M1	0.75	0.75
M2	0.5	1.0
M3	0.5	0.5
M4	1.0	1.0
M5	1.0	0.5

The coating procedure involved sequential deposition of a chitosan layer followed by a pectin layer. Initially, the liposomal suspension was added to the chitosan solution and incubated for 1 h, followed by refrigerated storage to ensure complete coating. Subsequently, the chitosan-coated liposomes were added to the pectin solution following the same procedure. After complete coating, the formulations were characterized in terms of mean size, ζ-potential, and PDI (Table 4).

Table 4. Characterization of coated liposomes – mean size, ζ – potential, polydispersity index

Model	Mean size ± SD (nm)	ζ-potential ± SD (mV)	Polydispersity index ± SD
M1	560.7 ± 4.1	-54.76 ± 0.12	0.81 ± 0.05
M2	2766.1 ± 3.4	-64.79 ± 0.45	0.99 ± 0.01
M3	829.2 ± 2.6	-52.69 ± 0.21	0.46 ± 0.04
M4	2275.3 ± 1.8	-41.08 ± 0.18	0.99 ± 0.01
M5	2674.8 ± 2.3	-62.61 ± 0.43	0.97 ± 0.02

The results demonstrated that the concentration ratio between chitosan and pectin strongly influenced the physicochemical properties of the coated vesicles. This behavior is consistent with previous studies on layer-by-layer coated liposomes, which demonstrated that increasing polysaccharide concentration may promote interparticle bridging and aggregation, leading to substantial increases in vesicle size and polydispersity [13, 21]. Formulations containing higher polymer concentrations showed a pronounced increase in particle size and PDI, suggesting the occurrence of particle aggregation and formation of larger polymeric complexes. This effect may be associated with excessive electrostatic interactions between oppositely charged polysaccharides leading to bridging phenomena between vesicles.

Among the tested formulations, M3 demonstrated the most favorable characteristics, exhibiting the lowest increase in mean

particle size while maintaining a relatively low PDI. These findings indicate successful deposition of the polymer layers without excessive aggregation of the liposomal system. Furthermore, the strongly negative ζ-potential values confirmed the successful deposition of the outer pectin layer onto the liposomal surface. Similar charge reversal following pectin deposition has been described in multilayer liposomal systems and is generally considered evidence of successful layer formation through electrostatic interactions between oppositely charged polymers [13, 21].

The obtained results suggest that optimization of polysaccharide concentration is critical for maintaining nanoscale dimensions and colloidal stability of layer-by-layer coated liposomes intended for oral delivery applications.

In vitro release of curcumin

The optimized coated liposomal formulation was subjected to *in vitro* release studies in order to evaluate the release profile of curcumin. The experiment was performed using the dialysis bag method in phosphate buffer pH 7.4 containing 20% methanol at 37°C.

The release profile demonstrated sustained release behavior, with approximately 70% of the encapsulated curcumin released over a period of 24 h (Fig. 1). The prolonged release profile may be attributed to the combined barrier effect of the lipid bilayer and the deposited polysaccharide coating, which likely slowed the diffusion of curcumin into the release medium.

The experimental data were fitted to several mathematical models describing drug release kinetics (Table 5).

Table 5. Drug release kinetics

Mathematical model	R^2 value
Zero-order	0.8089
First-order	0.9166
Higuchi	0.9522
Korsmeyer–Peppas	0.9367

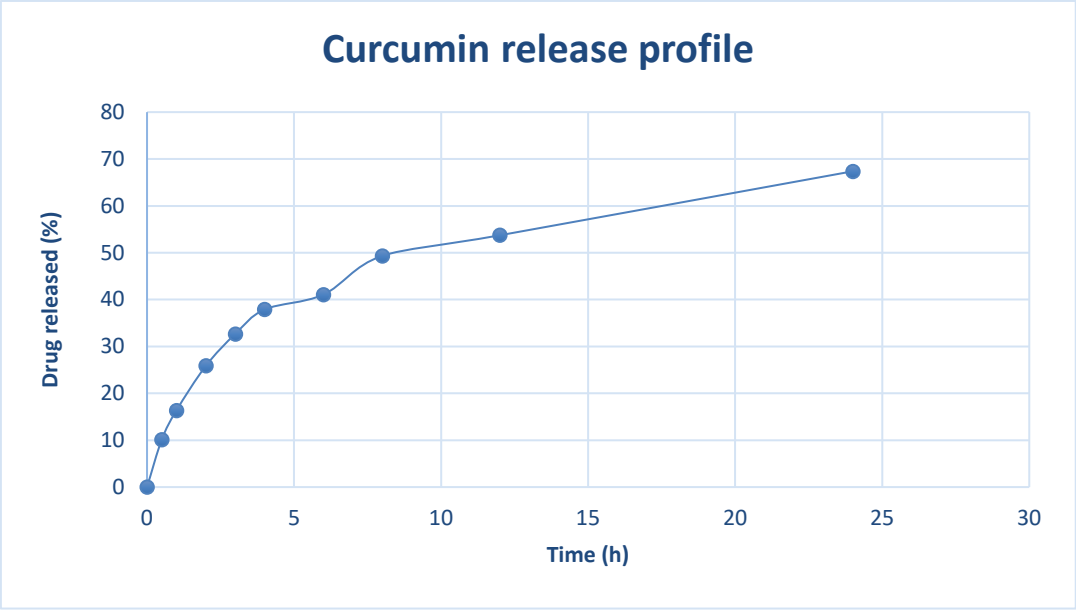

Figure 1. *In vitro* release of curcumin

The highest correlation coefficient was observed for the Higuchi model, indicating that curcumin release was predominantly governed by diffusion-controlled mechanisms. Such type of release behavior has frequently been reported for liposomal and polymer-coated nanocarriers, where the lipid bilayer and polymeric shell act as diffusion barriers controlling the release of the encapsulated compound [22, 23]. This finding is consistent with the structural characteristics of coated liposomal systems, where the encapsulated compound diffuses gradually through both the lipid bilayer and the polymeric coating layers.

Additionally, the good correlation with the Korsmeyer–Peppas model suggests that the release process may involve a combination of diffusion and polymer relaxation phenomena. The sustained release behavior observed in the present study is particularly advantageous for oral and colon-targeted delivery systems, as it may prolong drug residence time and improve local therapeutic activity within the gastrointestinal tract.

Conclusion

In conclusion, the ethanol injection method enabled the successful preparation of curcumin-loaded liposomes with relatively small particle size and favorable physicochemical characteristics. The sequential deposition of chitosan and pectin layers resulted in stable multilayer-coated liposomes without substantial increases in particle size under optimized conditions. Furthermore, the coated formulation demonstrated sustained in vitro release of curcumin, with release kinetics predominantly described by the Higuchi diffusion model.

Overall, the developed layer-by-layer coated liposomal system represents a promising strategy for improving the oral delivery and controlled release of curcumin, with potential applications in the treatment of inflammatory bowel diseases and other gastrointestinal inflammatory conditions.

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Simultaneous determination of cinnarizine and related substances in tablets by RP-HPLC

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Introduction: Cinnarizine is a cerebral blood flow improver. It is widely used orally for the treatment of cerebral apoplexy, cerebral arteriosclerosis and post-traumatic cerebral symptoms. It is also used for the control of nausea and vomiting. **Aim:** The aim of this study is to develop and validate accurate, precise and sensitive reverse-phase high performance liquid chromatographic method suitable to perform purity test of cinnarizine in tablets. **Materials and methods:** The chromatography was carried out on a base deactivated C18, 100 mm × 4.6 mm, 3 μm column, at a flow rate of 1.5 ml/min, with detection at 230 nm. Gradient elution mode was used for effective separation. The composition of mobile phases is as follows: mobile phase A – aqueous solution of ammonium acetate 10 g/l, mobile phase B – 0.2 v/v % solution of glacial acetic acid in acetonitrile. **Results and discussion:** This paper described the development of simple and reproducible analytical procedure for assay of cinnarizine and related substances in tablets by RP-HPLC. The method was validated for the parameters like specificity, linearity, precision, accuracy, LOD and LOQ. System suitability test was also performed that reveals effective separation between the drug of interest and flunarizine. **Conclusion:** The results of the studies showed that the proposed chromatographic procedure is rapid, precise and accurate, which can be applied for the routine assessment of cinnarizine in the presence of related substances in tablets.

Keywords: *RP-HPLC, validation, cinnarizine, impurities*

Introduction

Cinnarizine is piperazine derivative which is used as an antihistaminic and blood-flow promoter due to its H1-receptor and calcium-channel-blocking activity, respectively [1, 2]. It is recommended for the symptomatic relief of nausea and vertigo caused by Meniers and other vestibular diseases [3]. Several studies indicated that cinnarizine is highly efficacious in the treatment of motion sickness and has fewer side effects compared to other drugs [4].

Some analytical methods have been reported for assaying cinnarizine and related substances in pure as well as in pharmaceutical dosage forms. The methods include: high-performance liquid chromatography and thin layer chromatography [5–12], supercritical fluid chromatography [13], UV-spectrophotometry [14].

In our study, HPLC with UV detection and gradient elution was chosen for simultaneous determination of cinnarizine ant its impurities in the tablet dosage form.

Experimental

Chemicals and reagents

Cinnarizine and flunarizine reference standard was supplied from Sigma-Aldrich (Germany). HPLC grade acetonitrile and glacial acetic acid were produced from Merck. Cinnarizine tablets containing 25 mg active drug were commercially obtained.

Instrumentation and chromatographic conditions

Chromatography was carried out isocratically, on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 μl loop, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Compounds were separated on a base deactivated C18, 100 mm × 4.6 mm, 3 μm column, at a flow rate of 1.5 ml/min, with detection at 230 nm. Gradient elution mode was used for effective separation. The composition of mobile phases is as follows:

mobile phase A – aqueous solution of ammonium acetate 10 g/l, mobile phase B – 0.2 v/v % solution of glacial acetic acid in acetonitrile

Preparation of working solutions

Reference solution

25.0 mg of cinnarizine (reference substance) was dissolved in 100.0 ml volumetric flask with methanol. From the solution obtained, a solution with a concentration 6.25 μg/ml (0.25%) was prepared by dilution.

Sample preparation

Cinnarizine tablets were used for the investigation. One tablet contains 25 mg active substance. Twenty tablets were weighed and finely powdered. An amount of the powder equivalent to 250.0 mg of cinnarizine was transferred to a 100.0 ml volumetric flask. About 50 ml of methanol were added and the sample was shaken manually for 30 min. The sample was made up to the mark with methanol, mixed well and filtered. 20 μl of the filtrate was injected into chromatograph.

System suitability solution

12.5 mg of cinnarizine and 15.0 mg of flunarizine (standard substances) were dissolved in a volumetric flask of 100.0 ml with methanol. 1.0 ml of this solution was diluted to 20.0 ml with the same solvent.

Results and discussion

Validation of the method

The validation study allowed the evaluation of the method for its suitability for routine analysis. The proposed method was validated with respect to selectivity, linearity, precision and accuracy to show it could be used for simultaneous determination of cinnarizine and its related substances in the bulk drug and in pharmaceutical formulations.

The specificity of the HPLC method was confirmed by injecting blank samples and standard solutions. No other peaks were observed at the retention times of cinnarizine and its impurities, indicating that interfering substances were not present. The Fig. 1 showed typical chromatogram obtained from analysis of sample solution using the proposed method.

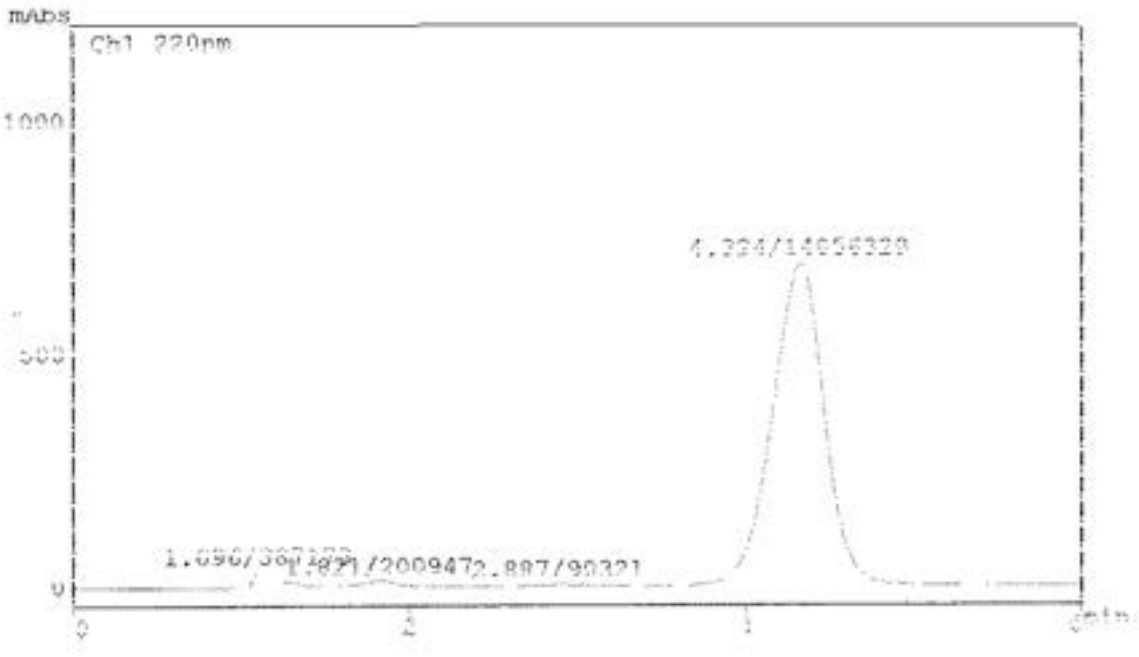

Figure 1. Chromatogram obtained from analysis of sample solution

Calibration and linearity

The linearity was tested in the range from 25% to 200% with respect to a standard solution of cinnarizine corresponding to 0.25% content of related substances. All the solutions were filtered through 0.22 μm membrane filter prior to use and injected in chrommatograph. For each step the solution was analyzed in triplicate. A calibration curve was plotted between the mean peak areas vs. respective concentrations. The corresponding linear regression equation was $y = 2111.5.x – 2030.5$ with square of correlation coefficient R^2 of 0.9999.

Precision

The precision of the method was evaluated by performing six independent determinations of the standard solution corresponding to 0.25% related substances and calculating RSD (%). The RSD values measured during assessment of precision was < 2.0%, confirming the method is precise (Table 1).

Table 1. Precision of the method

№	Amount claimed, %	Amount found, %
1.	0.25	0.248
2.	0.25	0.250
3.	0.25	0.248
4.	0.25	0.253
5.	0.25	0.251
6.	0.25	0.248
	Mean	0.250
	S_d	0.0021
	%RSD	0.839

Accuracy

Recovery studies were performed to validate the accuracy of developed method. The accuracy of the HPLC procedure was confirmed by adding cinnarizine reference substance in amounts of 50, 100 and 150% of the tested concentration (0.25%) to a constant amount of placebo.

Table 2. Statistical data for accuracy

№	Amount added, %	Amount recovered, %	Recovery, %
1.	50.0	50.52	101.0
2.		49.91	99.82
3.		50.61	101.2
1.	100.0	100.2	100.2
2.		99.73	99.73
3.		100.8	100.8
1.	150.0	149.6	99.73
2.		150.6	100.4
3.		148.6	99.07
		Mean:	100.2
		S_d	0.698
		RSD, %	0.697

Limit of quantification and limit of detection

The limit of detection (LOD) was calculated to be three times the standard deviation of baseline noise from analysis of each compound – 0.313 μg/ml. The limit of quantification (LOQ) was measured as the lowest amount of analyte that could be reproducibly quantified above the baseline noise, i.e. for each duplicate injection resulted in an RSD ≤ 2%. LOQ was found to be 0.625 μg/ml.

System suitability test

The suitability of the system was tested by chromatographing of a corresponding solution under described conditions. In the chromatogram resulted two well resolved peaks were observed with retention times 4.33 min for cinnarizine and 6.02 min for flunarizine, respectively. The test is valid if the separation between both peaks is at least 5.0.

Conclusion

Experimental results from validation studies showed that the proposed method is highly sensitive with satisfactory precision and accuracy. The developed RP-HPLC method was suitable for simultaneous

qualitative and quantitative determination of cinnarizine and its related substances in tablets and in raw materials.

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Microneedles as a modern transdermal drug delivery system

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Introduction: Transdermal drug delivery offers important advantages such as avoidance of gastrointestinal degradation and improved patient compliance. However, the outer layer of the skin represents a strong barrier that limits the penetration of many therapeutic agents. Microneedles have emerged as an innovative approach to overcome this limitation by enabling minimally invasive and effective transdermal delivery. **Aim:** The aim of this work is to review the principles, types, and pharmaceutical applications of microneedles as modern systems for transdermal drug delivery. **Materials and methods:** A literature survey was performed using scientific databases including PubMed, ScienceDirect, and Google Scholar, focusing on recent peer-reviewed publications on microneedle drug delivery systems. Different types of microneedles, including solid, coated, hollow, and dissolving systems, were examined with respect to their structure and mechanism of drug delivery. **Results and discussion:** Microneedles create micro-scale pathways in the skin that allow drugs to reach deeper layers without causing significant pain or tissue damage. They can improve the delivery of small molecules, peptides, and vaccines. Dissolving microneedles show particular promise due to their safety and ease of use. Despite these advantages, challenges remain regarding large-scale manufacturing and long-term stability. **Conclusion:** Microneedles represent a promising and patient-friendly technology for transdermal drug delivery. Continued research is needed to optimize their design and support broader clinical application.

Keywords: *microneedles, transdermal drug delivery, drug delivery systems, patient-friendly dosage forms*

Introduction

Transdermal drug delivery offers an alternative route of administration, allowing therapeutic agents to pass through the skin into the systemic circulation. This method provides several key advantages over traditional oral or parenteral drug delivery. For example, transdermal delivery can bypass gastrointestinal degradation and first-pass hepatic metabolism, which enhances the bioavailability of certain drugs. Additionally, transdermal systems can deliver drugs in a sustained and controlled manner, reducing dosing frequency and improving patient compliance, especially for managing chronic conditions (1, 2). However, the skin's barrier function significantly limits the effectiveness of transdermal drug delivery. The outermost layer, called the stratum corneum, plays a vital role in protecting the body from external physical, chemical, and biological threats. However, this highly organised layer also hinders the penetration of many therapeutic molecules. Consequently, only small, lipophilic drugs typically permeate the skin effectively, while hydrophilic compounds, macromolecules, peptides, and proteins tend to have poor transdermal absorption. To address these challenges, various enhancement strategies have been explored, such as chemical penetration enhancers, iontophoresis, sonophoresis, and microneedle-based delivery systems (3).

Among these methods, microneedles have gained significant scientific and clinical interest because they can create temporary microchannels in the skin in a minimally invasive and painless way (4). These tiny needle structures penetrate the outermost skin layer without reaching deeper nerve endings, allowing better drug delivery while ensuring patient comfort and safety. Microneedles can be fabricated from various materials, such as metals, silicon, and

biocompatible polymers, and can be designed in different shapes depending on the intended drug delivery method (5). Their flexibility allows for the administration of a broad spectrum of therapeutic agents, including small-molecule drugs, vaccines, peptides, and proteins. Recently, microneedle technology has also been explored for diagnostic and biosensing purposes, further expanding its potential role in modern medicine. As a result, microneedles are increasingly seen as a promising, patient-friendly approach for improving transdermal drug delivery and overcoming the limitations of traditional methods (5–7).

Aim

The aim of the present review is to summarise current knowledge regarding microneedles as modern systems for transdermal drug delivery. The article focuses on the principles of microneedle-mediated drug transport, the various types and materials used in their fabrication, and their pharmaceutical and biomedical applications. In addition, the review discusses the advantages, limitations, and future perspectives associated with the clinical implementation of microneedle-based delivery systems.

Methods

A comprehensive literature review was conducted to gather and analyse scientific information on microneedle-assisted transdermal drug delivery. Electronic databases, including PubMed, ScienceDirect, and Google Scholar, were systematically searched using keywords such as microneedles, transdermal drug delivery, microneedle fabrication, microneedle vaccines, and microneedle biosensors. Priority was given to recent peer-reviewed articles, review papers, and clinical studies published within the last 10 years to provide an up-to-date overview of current developments in the field.

Discussion

Types of microneedles

Microneedles can be classified into several categories based on their structural design and drug-delivery mechanism (Figure 1). These different categories have been developed to improve drug transport across the skin while minimising invasiveness and increasing patient acceptance (8). *Solid microneedles* are among the earliest designs and are mainly used to create temporary microchannels in the stratum corneum. This method, often called the “poke-and-patch” technique, involves first applying the microneedles to disrupt the skin barrier, then applying a drug formulation on the treated area. The drug then diffuses through the microchannels into deeper skin layers (9, 10). *Hollow microneedles* act as tiny hypodermic needles, allowing direct delivery of liquid drugs through an internal lumen. This design enables more precise dosing and quick drug absorption into the epidermis or dermis (11). However, making hollow microneedles is generally more complex, and issues such as clogging and fragility need to be addressed (12, 13). *Dissolving microneedles* have gained popularity because of their enhanced safety and ease of use. These are usually made from water-soluble or biodegradable polymers that contain the active drug inside the microneedle. When inserted into the skin, they gradually dissolve in the interstitial fluid, releasing the drug steadily without generating sharp medical waste (14–16). *Degradable microneedles* are designed for sustained or long-term drug release. Like dissolving types, they are made from biodegradable polymers but break down more slowly after insertion, enabling extended delivery of the medication. This makes them especially suitable for chronic conditions needing long-term treatment (17, 18). Recently, *bioresponsive microneedles* have been explored as advanced drug delivery

systems capable of releasing drugs in response to specific physiological signals (19). These signals might include changes in glucose levels, pH, temperature, or enzyme activity. Although bioresponsive microneedles show great promise for personalized and smart drug delivery, most remain in the experimental phase. Overall, the variety in microneedle designs supports the delivery of a wide range of therapeutic agents and drives ongoing innovation in transdermal drug delivery methods (20).

Fabrication techniques of microneedles

The successful application of microneedle systems in transdermal drug delivery is strongly dependent on the precision and reproducibility of their fabrication processes. Various microfabrication techniques have been developed to produce microneedles with appropriate geometry, mechanical strength, and drug loading capacity (21) (Figure 2).

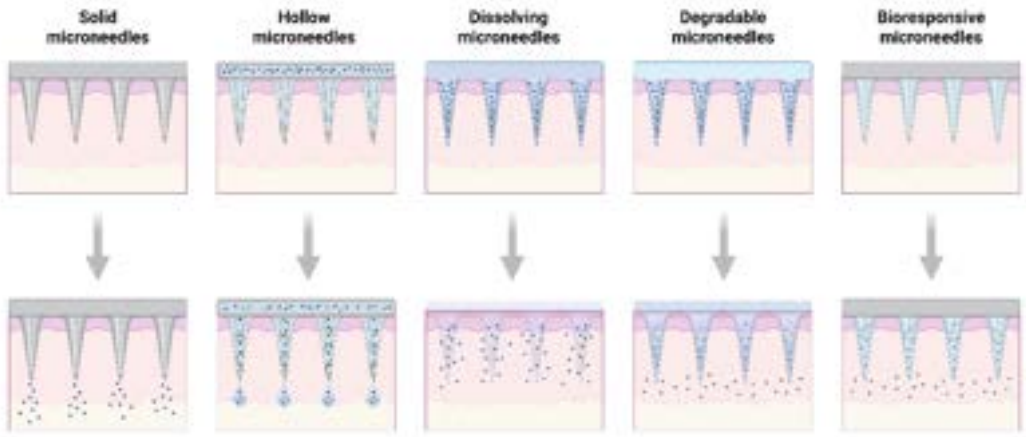

Figure 1. Delivery mechanisms through different types of microneedle patches. Created with BioRender.com (accessed on 27 April 2026).

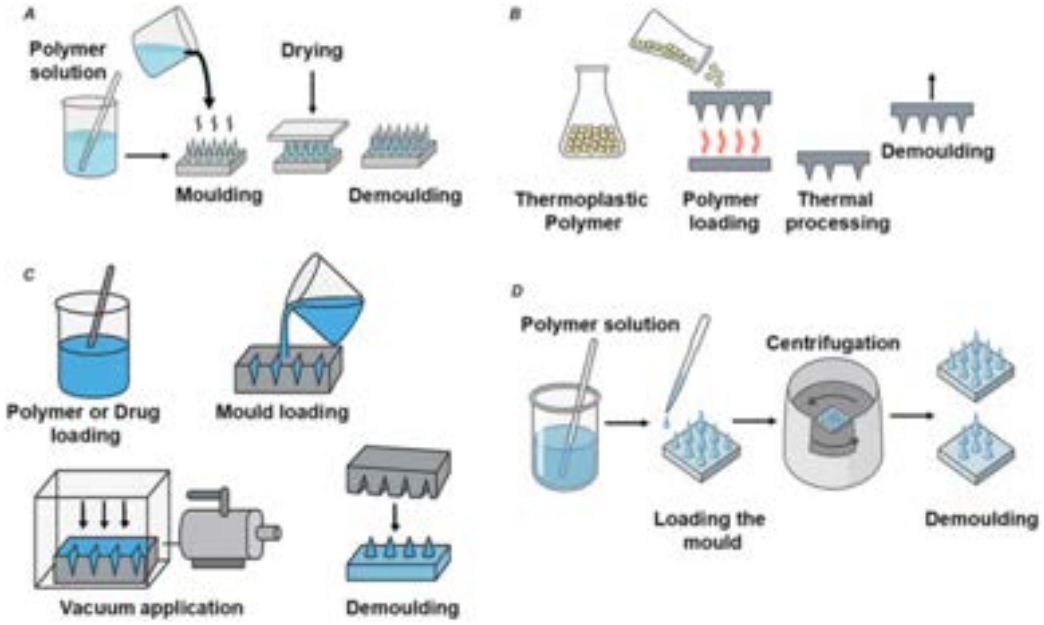

Figure 2. Schematic representation of common fabrication techniques for microneedle arrays. [Shah, S.W.A., Li, X., Yuan, H., Shen, H., Pan, G., Xie, H. and Shao, J. (2025), State-of-the-Art Fabrication of Microneedle Patches: A Mini-Review on Emerging Techniques. MedComm – Biomaterials and Applications, 4: e70020. https://doi.org/10.1002/mba2.70020] (21)

One of the most common fabrication methods is micromolding, especially for polymer-based dissolving microneedles. In this process, a polymer solution, often containing the active pharmaceutical ingredient, is poured into micro-scale molds with needle-shaped cavities. After solvent evaporation, drying, or curing, the solidified microneedle arrays are carefully removed from the molds. Micromolding provides benefits such as lower production costs, scalability, and the ability to incorporate drugs directly into the microneedle matrix (22).

Another important approach involves the thermal processing of thermoplastic polymers, in which materials such as biodegradable polymers are heated above their glass transition or melting temperature and shaped into microneedle structures using microstructured moulds. When cooled, the polymer maintains the desired microneedle shape. This method allows the fabrication of mechanically strong microneedles capable of reliable skin penetration (23).

To enhance mould filling efficiency and achieve uniform microneedle formation, vacuum-assisted fabrication techniques have also been developed. By applying reduced pressure, air bubbles can be removed, allowing the polymer solution to penetrate the mould's microcavities more effectively. Similarly, centrifugation-assisted micromoulding uses centrifugal force to push the formulation into the mould cavities, thereby improving structural uniformity and reproducibility (24).

Besides polymer-based manufacturing methods, other microfabrication techniques such as laser micromachining, photolithography, and injection moulding have been investigated, especially for creating metallic or silicon microneedles. The choice of a specific fabrication method depends on factors like the type of microneedle, material characteristics, drug formulation needs, and the requirement for large-scale production. Overall, advances in microneedle fabrication technologies have facilitated the development of versatile and customizable drug delivery systems with potential for industrial production and clinical use (21, 25).

Drug delivery mechanisms and pharmaceutical applications

Microneedle systems improve transdermal drug delivery by creating temporary micro-scale pathways across the stratum corneum. Depending on their design, microneedles can help transport drugs through different mechanisms, such as passive diffusion via microchannels, direct intradermal infusion of liquid formulations, or controlled release from biodegradable matrices (4). These mechanisms enable the delivery of a wide range of therapeutic agents that would otherwise have limited permeability through intact skin (26).

One of the most promising pharmaceutical applications of microneedle technology is vaccine delivery (27). The skin is rich in antigen-presenting cells, such as Langerhans cells and dermal dendritic cells, which can boost immune responses after antigen administration. Microneedle patches have been studied for delivering vaccines against influenza, measles–rubella, COVID-19, and other infectious diseases (28–30). This method could simplify immunization procedures, decrease the need for trained medical staff, and increase patient acceptance.

Microneedles have also demonstrated significant potential for the transdermal delivery of peptides and proteins, which are typically unsuitable for oral administration due to enzymatic degradation in the gastrointestinal tract (31). By bypassing the skin barrier, microneedle-based delivery can improve the bioavailability of these

biologically active molecules. Specifically, microneedle patches have been studied as alternative methods for insulin delivery in the management of diabetes mellitus, offering the possibility of less painful and more convenient long-term treatment (17).

In addition to systemic drug delivery, microneedles are widely studied for dermatological and cosmetic applications, including the treatment of acne, psoriasis, and skin aging (32–34). The controlled penetration of active substances into specific skin layers may enhance therapeutic efficacy while minimizing systemic side effects.

More recently, microneedle technology has been investigated for diagnostic and biosensing purposes, such as the monitoring of glucose levels and other biomarkers in interstitial fluid (35). The integration of microneedle arrays with wearable electronic devices represents a promising direction toward personalized and smart healthcare solutions. Overall, the versatility of microneedle systems supports their application in multiple therapeutic areas and highlights their potential as innovative platforms for modern drug delivery.

Future perspectives

Future research is likely to focus on developing advanced materials, smart bioresponsive systems, and wearable microneedle devices that combine drug delivery with real-time physiological monitoring. Integrating microneedle technology with nanotechnology and digital health platforms could further expand its uses in personalized medicine. Overall, although microneedle-based drug delivery systems show great promise, ongoing interdisciplinary research is crucial to overcoming current limitations and ensuring their successful transition from experimental studies to routine clinical practice (36).

Conclusion

Microneedle technology represents a promising and innovative approach for enhancing transdermal drug delivery. By enabling the creation of transient microchannels in the skin in a minimally invasive manner, microneedle systems facilitate the administration of a wide range of therapeutic agents that are otherwise difficult to deliver through conventional routes. Their potential applications extend from systemic drug delivery and vaccination to dermatological therapy and diagnostic monitoring.

Advances in microneedle design, materials, and fabrication techniques have contributed to the development of versatile delivery platforms with improved safety, patient acceptability, and therapeutic efficiency. Nevertheless, several challenges, including optimization of drug loading capacity, large-scale manufacturing, and long-term formulation stability, must be addressed to support broader clinical implementation.

Continued research and technological innovation are expected to further expand the role of microneedles in modern pharmaceutical and biomedical practice. In the future, microneedle-based systems may become an integral component of personalized and patient-centered therapeutic strategies.

Acknowledgement

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Advances in gene therapy for Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in substantia nigra, causing striatal dopamine depletion and hallmark motor symptoms. A central pathogenic mechanism is α-synuclein misfolding and aggregation, which contributes to synaptic dysfunction, neuroinflammation, and neuronal loss. Mutations in glucocerebrosidase (GCase) impair α-synuclein clearance, increasing PD risk. Genetic changes in leucine-rich repeat kinase 2 (LRRK-2) cause overactive kinase activity, disrupting neuronal processes. Current pharmacological therapies provide symptomatic relief but do not stop disease progression, underscoring the need for disease-modifying interventions. Gene therapy has emerged as a promising approach to address both motor symptoms and pathogenesis. This review summarizes advances in gene therapy for PD, focusing on molecular targets, vector platforms, and clinical outcomes. Gene therapy strategies can be categorized into dopamine restoration, neuroprotection, and disease modification. Dopamine-enhancing methods deliver genes encoding enzymes required for dopamine synthesis, including aromatic L-amino acid decarboxylase, tyrosine hydroxylase, and GTP cyclohydrolase 1. AXO-Lenti-PD exemplifies this approach, using a lentiviral vector to enable continuous dopamine production. Neuroprotective strategies focus on trophic factor delivery, such as glial cell line-derived neurotrophic factor, with AB-1005 designed to support neuronal survival. Disease-modifying approaches target α-synuclein and lysosomal dysfunction. CAP-003, a GCase-targeted gene therapy, aims to restore glucocerebrosidase activity and enhance α-synuclein clearance. Other strategies target LRRK-2 and intend to suppress excessive kinase activity. Adeno-associated viral and lentiviral vectors have demonstrated favorable safety and sustained transgene expression in clinical studies. Although efficacy has been modest, gene therapy remains a promising avenue toward disease-modifying PD treatments.

Keywords: *Parkinson's disease, gene therapy, dopamine restoration, neuroprotection, disease-modifying treatment.*

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. Its frequency significantly increases with age, making PD an age-related neurodegenerative disorder. It is characterized by bradykinesia accompanied by rigidity and resting tremors, which are consequence of the long preclinical phase in which we see progressive neuronal losses due to nigrostriatal dopaminergic degeneration [1]. However, PD is now considered a multisystem disorder with a wide range of non-motor symptoms like depression, anxiety, cognitive impairment, sleep and gastrointestinal disturbances, orthostatic hypotension [2]. The degeneration is not limited to the central nervous system, but it affects peripheral autonomic and enteric systems as well [3].

The primary pathophysiological feature is the degeneration of dopaminergic neurons in the substantia nigra, causing striatal dopamine depletion and hallmark motor symptoms. Misfolding and aggregation of α-synuclein contribute to synaptic dysfunction, neuroinflammation, and neuronal loss [2] (Fig. 1).

Current pharmacological therapies for PD provide symptomatic relief but do not stop its progression, underscoring the need for disease-modifying interventions. With the advancement of genomics and genetic treatment, new methods have emerged, addressing the problem that conventional therapies have been lacking. Gene therapies target the pathophysiology of the disease rather than the symptoms. This is done through enhancing the dopamine syntheses, delivering

neurotrophic factors, and modulating the processes of α-synuclein aggregation and lysosomal dysfunction [5]. Gene therapy has emerged as a promising approach to address both motor symptoms and pathogenesis. In the future, this might provide an opportunity to intervene earlier in the disease course and alter its progression.

Aim

This review summarizes advances in gene therapy for PD, focusing on molecular targets, vector platforms, and clinical outcomes.

Materials and methods

A literature review was undertaken using PubMed and Google Scholar databases. The following keywords and/or key phrases were used: “Parkinson’s disease”, “gene therapy”, and “mechanism”. The search identified a substantial number of articles, and publications from the past five years were subsequently assessed, with emphasis placed on systematic reviews and clinical trials.

Results and discussion

Gene therapy strategies can be categorized into dopamine restoration, neuroprotection, and disease modification (Fig. 2).

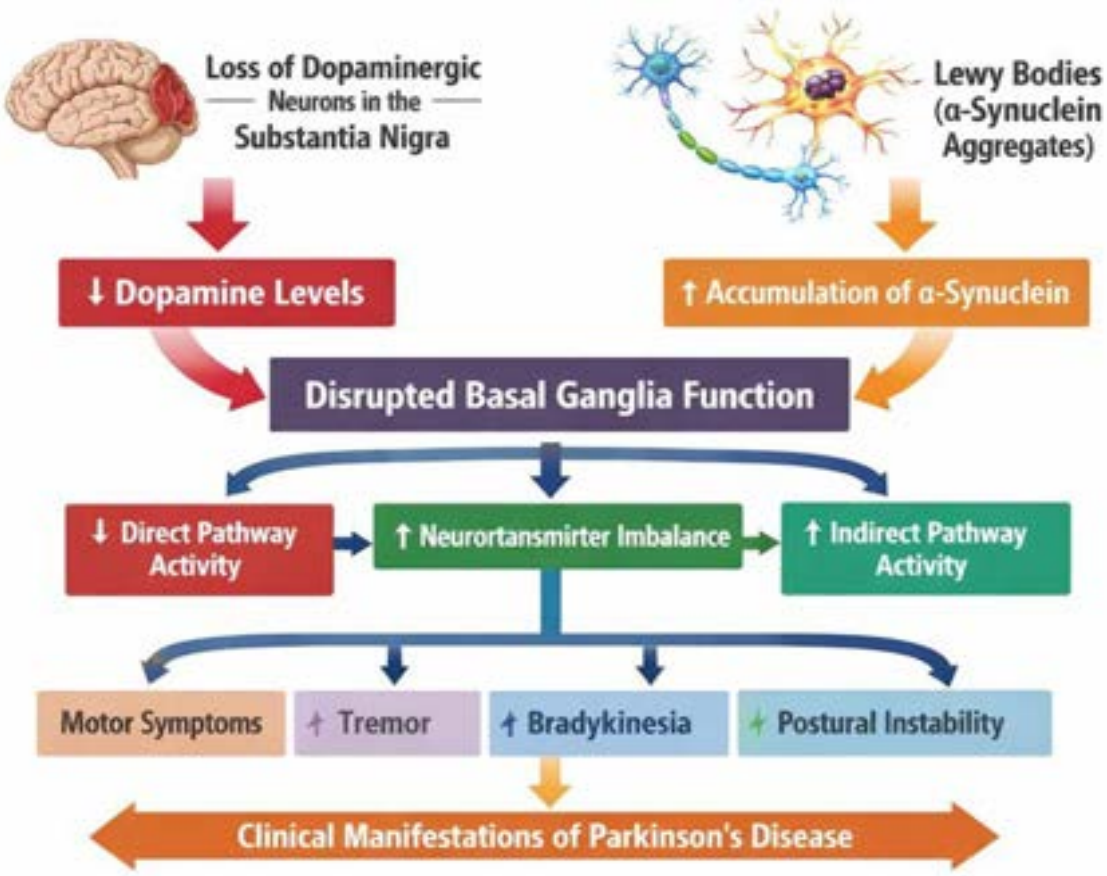

Figure 1. Pathophysiology of Parkinson’s disease.

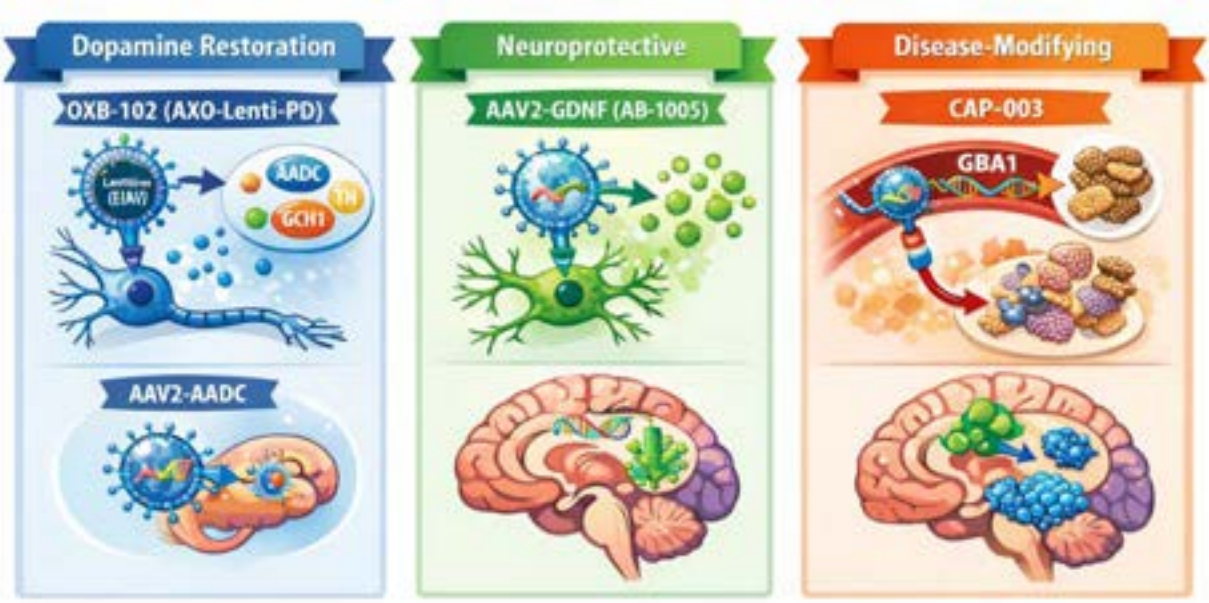

Figure 2. Gene therapy approaches in Parkinson's disease.

1. Dopamine restoration

Dopamine-enhancing methods deliver genes encoding three enzymes required for dopamine synthesis: aromatic L-amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), and GTP cyclohydrolase 1 (GCH 1). The interdependence of these enzymes is essential. Disruption of any component compromises the entire pathway:

- AADC catalyzes the decarboxylation of L-DOPA to dopamine. With the advancement of PD and the progressive degradation of neurons, AADC activity is significantly reduced, leading to no responsiveness to exogenously administered L-DOPA [6].
- TH converts tyrosine to L-DOPA. Without TH, dopamine production depends on exogenous L-DOPA.
- GCH1 synthesizes tetrahydrobiopterin (BH4), a cofactor for TH. In the absence of BH4, TH is inactive. Thus, GCH1 indirectly regulates dopamine production by sustaining TH activity [7].

Dopamine restoration therapy is based on restarting the dopamine synthesis directly within the striatum. This is achieved by genetically modifying neurons to express key enzymes of the dopamine biosynthetic pathway, enabling local dopamine production independent of the nigrostriatal system [8]. This method does not restore the lost neuronal pathways but rather bypasses them, which makes that method symptomatic rather than disease modification [8, 9].

1.1. OXB-102 (AXO-Lenti-PD)

OXB-102 (AXO-Lenti-PD) exemplifies a dopamine restoration approach, using a lentiviral vector to enable continuous dopamine production. This method uses an equine infectious anemia virus (EIAV) to deliver the genes for all three enzymes simultaneously. The vectors integrate into the host genome, making long-term expression of transgenes possible. These genes work together so that the transduced neurons can produce dopamine inside the brain on their own. Because the genes are permanently integrated into the DNA of the targeted cells, a single administration is intended to provide lasting enzyme expression and dopamine production, potentially reducing the need for frequent oral medications. Disadvantages of this

therapy are the lack of effect on the pathophysiology of PD, α-synuclein aggregation, neuroinflammation, and neuronal loss. Permanent genome integration creates potential safety concerns, namely mutagenesis. And last but not least, the procedure is complicated and carries underling procedural risks [7].

1.2. AAV2-AADC

AAV2-AADC uses adeno-associated virus (AAV) serotype 2 (AAV2) as a delivery vehicle. It improves dopamine synthesis by increasing AADC production in the basal ganglia. According to clinical studies, the introduction of AAV2-AADC shows encouraging results because it is low immunogenic and can significantly improve motor symptoms and reduce dependence on dopaminergic drugs. However, AAV2-AADC gene therapy does not have long-term effectiveness over time. There is a risk that the AADC enzyme will be degraded or the AAV vector will lose its activity. It is possible that the small immune response will affect the results of the therapy in the long term and may lead to adverse reactions such as inflammation [10].

2. Neuroprotection

Neuroprotective strategies focus on trophic factor delivery, such as glial-derived neurotrophic factor (GDNF).

Gene therapy with AAV2-GDNF (AB-1005) introduces the GDNF gene into the brain cells. This gene is known to enhance neuronal growth and protect against neurotoxicity and prevent the degeneration of dopaminergic neurons. AAV2-GDNF is designed to support neuronal survival, protect or restore dopamine related neurons – rather than just replacing dopamine like standard medications. However, it has variable clinical efficacy, limited distribution in brain tissue, and requires invasive intracranial delivery. Unlike pre-clinical studies, clinical trial results are not as good. Despite GDNF introduction, motor function does not always improve because it is not delivered to the right location. Researchers are focusing on exploring combination therapies and improving delivery methods to improve the effectiveness of AAH2-GDNF therapy [11].

3. Disease modification

Disease modification in PD aims to directly interfere with the pathogenic mechanisms of neurodegeneration. These approaches target α-synuclein aggregation, lysosomal dysfunction, and kinase dysregulation [12]. Those pathways are interconnected and form a system in which impaired protein clearance, toxic protein accumulation, and disrupted intracellular signaling simultaneously contribute to disease progression [13].

3.1. α-synuclein targeting

There are different strategies that target α-synuclein: using gene silencing, such as antisense oligonucleotides or RNA interference, to limit the production of the protein at the transcriptional or translational level [12, 13]; to stimulate autophagy, ensuring effective degradation and clearance of α-synuclein from the neurons [14], and last but not least, to use immunotherapy to target the extracellular α-synuclein [15]. A lot of those approaches have progressed to early-stage clinical trials whose results are contradictory. A major disadvantage of α-synuclein targeting is the fact that the protein has other roles like synaptic function and neurotransmission, making complete suppression undesirable. Thus, the conclusion is reached that it has to be carefully modulated rather than inhibited completely [12].

3.2. Improvement of lysosomal function

There is strong support for this therapy, because it addresses one of the biggest risk factors for PD – mutations in the GBA1 gene, which leads to impairment in the activity of lysosomal enzyme GCase, and are associated with the most aggressive disease progression. GCase degrades cellular substrates, and its dysfunction results in the accumulation of α-synuclein [16]. Novel therapeutic strategies try to restore GCase activity by delivering copies of the GBA1 gene to affected neurons, normalizing lysosomal function, and improving α-synuclein clearance [13].

An example of this approach is CAP-003, which is designed to target a specific genetic subtype of PD associated with GBA1 gene mutations. CAP 003 uses an AAV vector and carries a functional copy of the GBA1 gene. Subsequently, it restores GCase activity, improves lysosomal function, and enhances clearance of α-synuclein aggregates [13]. CAP 003 is intended to be given as a single intravenous infusion, not injected directly into the brain like many other gene therapies. It crosses the blood–brain barrier, reaching many brain regions [17]. However, CAP 003 has its limitations, the most important of which is that it can only be used on patients with the GBA1 mutations, and this fact limits its relevance to a specific genetic subset of PD rather than the broader population [13].

3.3. LRRK2 inhibition

Parkinson's disease linked to mutations in LLRK2 gene is manifested by muscle rigidity, tremor, and bradykinesia [18]. They are autosomal dominant variants that lead to excessive kinase activity, neuronal dysfunction, and often to the development of the disease. The LLRK2 gene is part of the leucine-rich repeat kinase family and encodes many domains, such as a RAS-related domain, a GTPase domain, and a serine/threonine domain. Over the years, many targeted therapies for LRRK2 suggest ways to modify the genome and intend to suppress excessive kinase activity. Instances of this approach are zinc finger nucleases and helper-dependent adenoviral vectors [18, 19].

Conclusion

Adeno-associated viral and lentiviral vectors have demonstrated favorable safety and sustained transgene expression in clinical studies. Although efficacy has been modest, gene therapy remains a promising avenue toward novel PD treatments.

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New directions in the pharmacotherapy of epilepsy: a review

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Epilepsy is one of the most common chronic neurological disorders, affecting more than 70 million people worldwide. The clinical manifestation of epileptic seizures depends on the location of cortical discharges and their spread within the brain. Seizures and epilepsy are the consequence of an imbalance between excitation and inhibition within certain regions of the central nervous system. According to the International League Against Epilepsy, this disorder can be classified as genetic, structural, metabolic, infectious, autoimmune, or of unknown origin. An essential component of successful antiepileptic treatment is providing good seizure control with fewer adverse effects while maintaining a high quality of life. This review aims to summarise the current evidence and recent developments in antiepileptic treatment strategies. Due to differences in the mechanisms of action of anti-seizure medications (ASMs), choosing the most appropriate antiepileptic treatment is important. Monotherapy with first-line ASMs (Valproate, Carbamazepine, Ethosuximide, Lamotrigine, and Topiramate) is initiated, depending on the type of epilepsy and the type of seizures. If initial therapy proves ineffective or is poorly tolerated, monotherapy with an alternative second-line ASMs is recommended. Patients with drug-resistant epilepsy may require polypharmacy or even neurosurgical intervention. In recent years, the development of novel ASMs has expanded, including cenobamate and fenfluramine, which have demonstrated significant therapeutic potential. Ongoing advances in epilepsy genetics and the discovery of novel drug mechanisms have led to the development of more than 200 new therapeutic approaches, including gene therapies, which are currently undergoing preclinical or clinical evaluation.

Keywords: *epilepsy, ASM, gene therapy, preclinical and clinical data*

Introduction

Epilepsy is a chronic neurological disorder characterized by abnormal neuronal activity that leads to repeated and unpredictable changes in brain function (1). Approximately 4.9 million new cases of epilepsy are diagnosed every year (2). The disorder manifests as a variety of symptoms, including convulsions, loss of consciousness, muscle stiffness, and prolonged muscle contractions. These symptoms may significantly impair quality of life (3). An epileptic seizure is a sudden disturbance of consciousness and of sensory, motor, and mental functions. A diagnosis is based on the occurrence of at least two unprovoked seizures.

Seizures vary in their localization and dispersion within the brain:

1. Focal – confined to one hemisphere.
2. Generalized excitation originates in a particular region and disseminates through neural networks; it may exhibit asymmetry.
3. Indeterminate onset.

In numerous instances, the long-term outlook for epilepsy is adverse. Patients exhibit an elevated risk of developing other systemic and mental comorbidities, along with an increased death risk. Antiepileptic therapy aims to improve quality of life by reducing seizure frequency. However, in some cases, it can worsen symptoms due to serious side effects (4). Epidemiology – in our nation, over 50,000 individuals are affected. Epilepsy commences in 50–60% of patients before the age of 16, while febrile seizures manifest in 2–5% of children under 5 years old (4).

Epilepsy has a multifactorial etiology involving complex mechanisms, including abnormal neuronal activity, an imbalance of neurotransmitters, ion channel dysfunction, and genetic predisposition. Inflammation,

oxidative stress, and immune responses are additional factors that contribute to the onset and progression of the condition (5). Over 30 antiepileptic medications (AEMs) have received approval for treating epilepsy. These drugs work by modulating voltage-gated ion channels, enhancing GABA-ergic transmission, and inhibiting glutamate receptors, among other mechanisms. Despite these advances, approximately 30% of patients have drug-resistant epilepsy. In this case, monotherapy is insufficient, and polypharmacotherapy is required, which involves the simultaneous use of two to four AEMs with complementary mechanisms of action (6).

Classification of epilepsy according to its etiology (7):

- Genetic epilepsy – primarily triggered by genetic factors or mutations.
- Structural epilepsy – linked to physical abnormalities in the brain (e.g., tumors, stroke, trauma, cortical malformations.
- Metabolic epilepsy – caused by metabolic disturbances that impact brain function.
- Immune epilepsy – connected to autoimmune or inflammatory reactions.
- Infectious epilepsy – brought about by infections in the central nervous system.
- Unknown etiology epilepsy – when the cause cannot be determined.

Diagnosis of epilepsy:

- Electroencephalography (EEG) – used for diagnosing epilepsy and monitoring the effect of treatment. The absence of EEG abnormalities does not exclude epilepsy, while their presence supports the diagnosis.
- Computed tomography (CT) with contrast – mainly used in emergency cases and for detecting larger structural abnormalities.
- Magnetic resonance imaging (MRI) – a mandatory, safe, and effective method for early detection of drug-resistant epilepsy and for prognosis (8).
- Laboratory tests.

The aim of this review is to summarize the available data and latest trends in pharmacotherapeutic approaches and treatment strategies for epilepsy.

Treatment

The objective of treatment is to achieve complete seizure control while minimizing adverse effects and maximizing quality of life. The standard approach to epilepsy treatment involves pharmacotherapy, which can be administered as monotherapy or polytherapy.

When pharmacotherapy is ineffective or contraindicated, neurosurgical interventions or alternative therapeutic modalities may be employed, including ketogenic diet therapy and vagus nerve stimulation. It is crucial to begin treatment only after a definitive diagnosis has been established. It is important to note that treatment should not be started following a single seizure, regardless of whether it is accidental or symptomatic (9). Additionally, treatment should not be initiated if there are no electroencephalographic (EEG) abnormalities present.

The fundamental principles of epilepsy treatment can be categorized as follows:

1. The initial step in evaluating patients with epilepsy is a comprehensive assessment to determine the type of epilepsy and seizure, as well as the risk of pharmacological therapy.

2. Treatment always begins with a low dose of a first-line drug. The specific drug is chosen based on the type of seizures, the patient's age, and any comorbid conditions.

3. Monitoring of serum drug levels is also essential.

Monotherapy has been demonstrated to be effective in 75%–80% of cases. For drug-resistant epilepsy, polytherapy is often necessary. However, this approach carries a risk of serious adverse reactions due to metabolic changes and drug-drug interactions (6).

Figure 1. History of antiepileptic drug development.

❖ **First-line monotherapy drugs are:**

- *Valproate (VPA)* is a medication used to treat two forms of epilepsy: generalized and focal. Studies have demonstrated that the substance can cause teratogenic effects in young women of reproductive age and during pregnancy.
- *Carbamazepine (CBZ)* is employed in the treatment of focal epilepsies, while oxcarbazepine is utilized in cases of childhood-onset epilepsy.
- *Ethosuximide (ESM)* is the preferred medication for treating absence seizures.
- Broad-spectrum drugs, including valproate, lamotrigine (LTG), levetiracetam (LEV), and topiramate (TPM), are considered first-line treatment options for patients exhibiting multiple seizure types.
- *Lamotrigine and oxcarbazepine (OXC)* can be used as monotherapy in children and adolescents, young women, pregnant patients, and in individuals with cognitive impairment (1).

❖ **Second-line monotherapy drugs** are used when there is no response to treatment, intolerance, or serious side effects. In these cases, an alternative medication is administered and the initial drug is gradually discontinued. Lamotrigine (LTG), levetiracetam (LEV), and topiramate (TPM) are used for generalized and focal seizures, with or without secondary generalization. The recommended treatment for focal and secondarily generalized seizures is oxcarbazepine (OXC) (10).

Antiepileptic drugs can also be classified according to their mechanism of action:

- The modulation of voltage-gated ion channels (Na, K, and Ca) is a critical aspect of pharmacological research. Notable examples of compounds that have been studied in this context include phenytoin, carbamazepine (CBZ), and lacosamide (11).
- The enhancement of GABA-mediated inhibitory neurotransmission has been observed in the presence of phenobarbital (PB) and benzodiazepines (BZs) (10).
- A reduction in glutamate-mediated excitatory neurotransmission has been observed in response to perampanel, – an AMPA receptor antagonist.
- Inhibition of synaptic vesicle protein

2A (SV2A) modulates presynaptic neurotransmitter release – levetiracetam.

- Innovative drugs aimed at metabolic and signaling networks, including the mTOR (mammalian target of rapamycin) inhibitor everolimus.
- Multitarget therapy – metabolic-immune modulators: metformin enhances mitochondrial activity and inhibits the activation of the NLRP3 inflammasome.
- Modulators of transient receptor potential vanilloid 1 (TRPV1): cannabidiol (CBD) may augment inhibitory effects and operates through dual targeting of TRPV1 and GPR55 (G-protein-coupled receptor 55) (12).

❖ **New directions in the treatment**

Gene therapy represents an important new step in the treatment of epilepsy, as it is based on the concept of targeting genetic determinants rather than merely alleviating symptoms. This approach involves directly delivering therapeutic genes to neuronal cells to modify neural circuits and reduce seizure frequency. The techniques currently used include various delivery methods such as viruses (most commonly adeno-associated viruses (AAV), lentiviruses, and herpes simplex viruses) and nanoparticles (13). To date, viral vectors are the most frequently used (14). After intracerebral administration and crossing the blood-brain barrier, a vector containing genetic material can lead to correction of the existing genetic defect (15).

Currently, gene therapy is recommended only for individuals with no other treatment options, including surgical interventions. Despite novel therapeutic strategies, pharmaceutical intervention remains the cornerstone of seizure management. New drugs approved by the EMA over the past 5 years (16):

- *Cannabidiol* (2019) – Its mechanism of action involves reducing neuronal hyperexcitability by interacting with GPR55 and TRPV1 receptors, as well as by suppressing cellular adenosine uptake via nucleoside transporter 1 (ENT-1). It is used solely as a supplementary therapy. Reported common side effects include diarrhea, somnolence, and elevated liver enzymes (17).
- *Fenfluramine* (2020) – is a substance similar to cannabidiol that is used as an add-on treatment. It acts by activating the serotoninergic receptors 5-HT1D, 5-HT2A, and 5-HT2C, as well as the serotonin-1 receptor. This activity is thought to restore the balance between excitatory and inhibitory systems, thereby reducing seizure frequency. Common side effects include cardiovascular complications. One advantage is its beneficial effect on cognitive function and attention in patients treated with it (18).

Conclusion

Treating epilepsy remains one of the most challenging issues in modern medicine. Despite progress in the efficiency of existing methods, they still seem inadequate for many patients. Conversely, the introduction of new antiepileptic drugs and gene therapy has sparked a dynamic shift in therapeutic strategies, and these treatments are expected to play an increasingly important role in epilepsy treatment.

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Current understanding of endometriosis: review of its drug treatment

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Endometriosis is a chronic estrogen-dependent inflammatory disease affecting approximately 10% of reproductive-age females. The exact cause of endometriosis is unclear, but theories include retrograde menstruation, genetic factors, and immune system dysfunction. The major symptoms are chronic pelvic pain, dysmenorrhea, and infertility. This review aims to present the current medications used to manage endometriosis and future therapeutic directions. First-line standard treatments consist of NSAIDs, combined contraceptives, and progestins, while gonadotropin-releasing hormone (GnRH) agonists or antagonists and aromatase inhibitors are reserved for resistant cases. Hormonal medications work by suppressing ovarian function, inhibiting estrogen production, or creating a state similar to pregnancy or menopause. GnRH agonists initially stimulate the release of gonadotropins, leading to an increase in estrogen production. However, with prolonged use, they cause a downregulation of GnRH receptors, which decreases estrogen levels and suppresses ovarian function and reduces the size of endometriotic lesions. GnRH antagonists, however, directly block the GnRH receptors, resulting in a rapid and reversible suppression of ovarian function and a decrease in estrogen levels. Both types of medication are generally used for short-term treatment due to side effects and their potential impact on bone health. Progestins have been shown to inhibit the progression of endometriosis effectively. Combined hormonal contraceptives have been found to be effective in reducing dysmenorrhea and pain, but the long-term use of combined contraceptives also has negative effects. Advances in genomics enable personalized treatment, including novel targeted therapies such as tumor necrosis factor inhibitors for endometriosis.

Keywords: *endometriosis, inflammation, hormonal therapy, new targeted therapies*

Introduction

Endometriosis is a disorder in which tissue resembling the lining of the uterus develops in locations outside the uterine cavity (1). It is seen in a considerable number of women during their reproductive years and is a common reason for pelvic pain and difficulties with fertility (2). Even though it is rather common, the diagnosis is often set back, which can lead to discomfort and reduced quality of life (3). The way the disease presents can differ significantly from one patient to another; some women may not notice clear symptoms, while others experience intense menstrual pain, pelvic discomfort, pain during sexual intercourse, or problems with conception (4). In many cases, the intensity of symptoms does not reflect the extent of the disease, which makes both diagnosis and treatment more complicated. Apart from physical complaints, the condition can also affect emotional well-being, daily functioning, and overall productivity (5). The exact cause of endometriosis is still not completely clarified; it is believed that several factors are involved, including menstrual reflux, genetic background, hormonal influences, and changes in immune function (6). A key feature of the disease is its reliance on estrogen, which supports the survival and growth of endometriotic tissue. Because of this, most treatment approaches are aimed at lowering estrogen levels or reducing its effects. At present, there is no therapy that can permanently cure endometriosis (7). Surgical procedures can remove visible lesions, but they do not guarantee that the disease will not return, for this reason, medical treatment plays a major role in long-term control. The main goals are to relieve symptoms, slow down

disease progression, and improve the patient's quality of life (8). Since endometriosis tends to recur, treatment decisions must consider effectiveness, possible side effects, and future fertility plans (8). With the ongoing development of new medications and more targeted therapies, it is important to continuously reassess current treatment strategies (9). This review looks at commonly used medical treatments as well as newer options that are being introduced (9).

Pathogenesis of endometriosis

The pathogenesis of endometriosis is complex and still not fully understood. The most widely accepted and supported hypotheses include retrograde menstruation, the stem cell theory, and immunological factors.

1. Retrograde menstruation theory

The most widely accepted theory is Sampson's theory of retrograde menstruation. According to this theory, during menstruation, part of the menstrual fluid containing endometrial cells flows backward through the fallopian tubes into the pelvic cavity rather than leaving the body. These displaced cells can attach to pelvic tissues, proliferate, and form endometriotic lesions (10).

2. Stem cell theory

According to this theory, endometrial stem cells that are derived from bone marrow or the endometrium can migrate to ectopic locations and differentiate into endometrial tissue. Endometrial stem cells may also be shed in the blood during menstruation and then enter a new location in the body, where they can form DII or other forms of endometriosis, such as pulmonary endometriosis (11).

3. Immunological factors

The pathogenesis of endometriosis has also been attributed to immunological factors, as several studies have observed immunological changes in women with endometriosis, including reduced natural killer (NK) cell function and elevated levels of inflammatory cytokines (12, 13).

Aim

The aim of this review is to outline the main drug-based treatment options for endometriosis, including both established therapies and newer approaches. The focus is on their mode of action, clinical benefits, and potential limitations.

Materials and methods

The search strategy was directed towards studies relating with treatment of endometriosis. An expanded search for articles from the last five years was conducted on the following databases: Google Scholar, PubMed, Scopus, and Web of Science. In the search process, the following key words were used: "*endometriosis*", "*new therapies*", "*pathogenesis*", "*treatment*".

Results

1. Drug treatment

1.1. Nonsteroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs have many mechanisms of action. The first one is inhibiting the cyclooxygenase enzymes and blocking the pathway, leading to pain and inflammation. Another one is directly affecting the nociceptive receptors, which causes a block of releasing pain-causing substances (1, 2) (Fig. 1). However, while effective for dysmenorrhea, their prolonged use can lead to various side effects. They are divided into gastrointestinal ones, such as dyspepsia, heartburn and even the appearance of ulcers, renal ones, including acute, and chronic renal failure and cardiovascular ones, for example, an increased risk of hypertension (14).

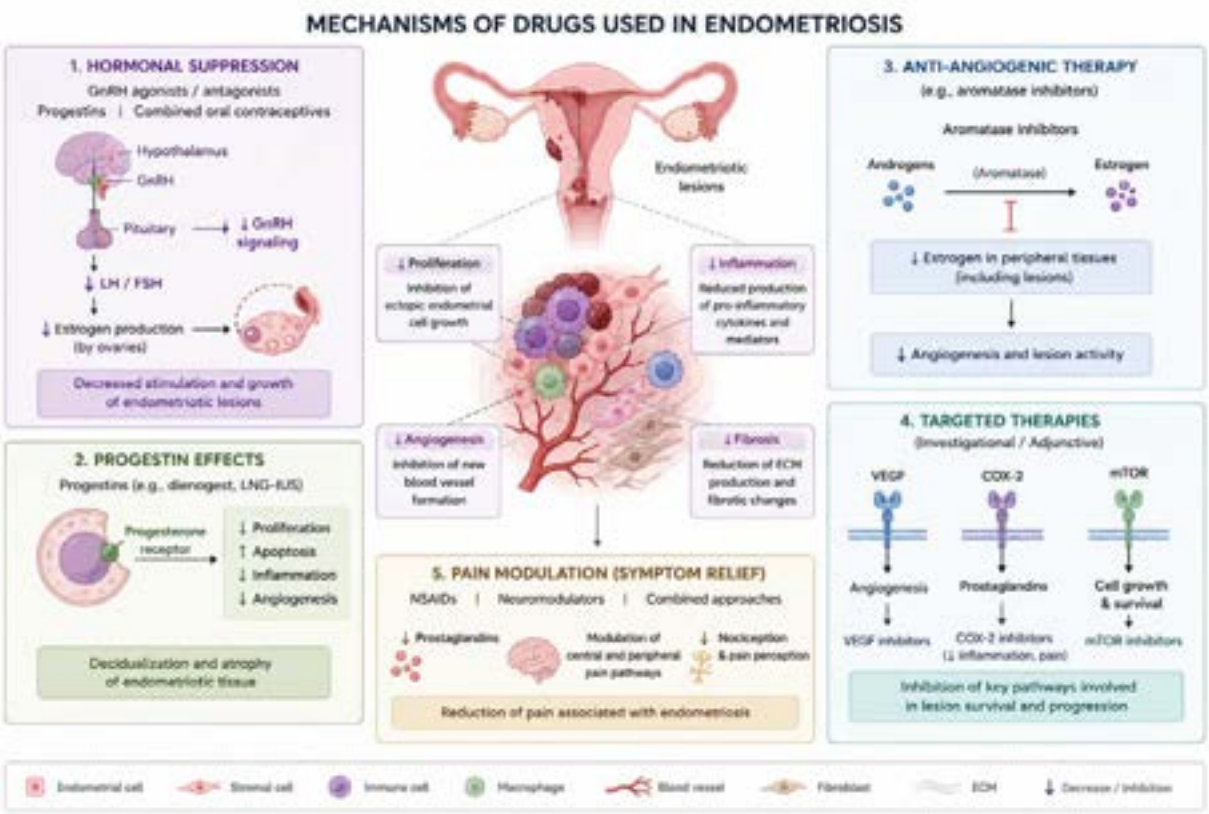

Figure 1. Mechanisms of action of drugs used in endometriosis.

1.2. Hormonal therapies

Progesterone drugs have more than one mechanism of action, some of which include preventing the proliferation of endometrial cells, and inhibiting the inflammation process, which has crucial role in the evolution of endometriosis (1) (Fig. 1). The progestin-only therapies include medroxyprogesterone, norethisterone and dienogest (2). Their side effects include gaining weight, fluid retention, breast tenderness, and bleeding of the genital tract outside of menstruation (15).

Combined oral contraceptives are usually used as first-line treatment. They are mainly used for alleviating symptoms like dysmenorrhea, because of their ability to suppress ovulation, which leads to stabilizing the endometrium (3). They are a good option, because they are affordable and yet effective if taken properly (16).

1.2.1. GnRH agonists and antagonists

GnRH antagonists are a newer way to manage symptoms by directly blocking the receptors in the pituitary gland (12) (Fig. 1). The major advantage is their rapid onset of action by suppressing LH and FSH immediately, avoiding the temporary “symptom flare” that older agonists often cause (15).

Oral versions, in particular, offer more flexibility – they allow for “partial” estrogen suppression (17). This is key because it can help manage the pain while keeping those harsh, low-estrogen side effects (like hot flashes) more manageable (18). While they are great for pain relief, they do require a long-term eye on bone density and heart health (16, 19).

GnRH agonists have been a standard treatment for years, they work by overstimulating the pituitary gland, which eventually

causes it to stop producing the hormones (LH and FSH) that trigger estrogen production (20). The main thing to know about agonists is the “flare effect” because they start by stimulating the gland, there’s usually a temporary spike in estrogen levels during the first week or two, which can actually make symptoms feel worse before they get better. After that initial phase, they create a state of “medical menopause” to starve the endometriosis of estrogen (21).

Because the drop in estrogen is so significant, these are often paired with “add-back therapy” (a low dose of progestin or estrogen) to protect bone density and reduce side effects like hot flashes and night sweats, they are highly effective at shrinking lesions, but because of their intensity. They are typically used for shorter, defined periods of time (22).

1.2.2. Aromatase inhibitors

Aromatase inhibitors (AIs) work differently by blocking the conversion of androgens to estrogen by the body (23). This is especially useful for endometriosis, as the displaced tissue can actually produce its own estrogen locally, essentially feeding its own growth (24) (Fig. 1). AIs such as Letrozole and Anastrozole can effectively shrink growth and reduce pain by lowering estrogen both throughout the body and specifically at the lesion sites, especially for patients who haven't had luck with other treatments (2). But because they reduce estrogen so much, they can sometimes cause stiff joints or loss of bone density, which usually limits how long they can be used (15).

1.2.3. Progestins and oral contraceptives

Treatment of endometriosis often involves the use of progestins (norethindrone acetate, dienogest) and oral contraceptives (24, 25) (Fig. 1). A systematic review reported that oral contraceptives and progestin-only pills are effective in decreasing pain symptoms and improving quality of life in women with endometriosis. That review did not find sufficient evidence to recommend one hormonal approach over another (24). However, treatment decisions should be individualized based on patient symptoms, preferences, and clinical characteristics, and further research is necessary to determine the optimal treatment strategies for different patient subgroups.

1.3. New targeted therapies

Recent advances in the medical treatment of endometriosis have focused on developing new targeted therapies. These approaches aim to specifically affect the main mechanisms involved in the development of endometriosis, which may provide more effective treatment options. Some promising targeted therapies include anti-inflammatory agents, selective estrogen and progesterone modulators, statins, antiangiogenic compounds, stem cell-based therapies (26) (Fig. 1), and immune system modulation to ease the immune response. There is also potential for using gene-based and epigenetic therapies to “reset” how the body handles hormones (27). However, despite these encouraging early results, current research is still mostly experimental and has mainly been conducted in animal models (28).

2. Surgical management

Laparoscopy is the recommended surgical technique for the treatment of endometriosis regardless of its stage. Conservative surgical management is preferred in women of reproductive age who plan to conceive. The best therapeutic outcomes are achieved through a combination of surgical and pharmacological treatment (15).

Discussion

Current pharmacological approaches to endometriosis mainly focus on controlling symptoms and reducing disease activity rather than finding a definitive cure. Most available treatments work by lowering estrogen levels or altering its effects. This results in decreased growth of endometriotic lesions and relief from pain symptoms (2). Hormonal therapies are the mainstay of treatment. Combined hormonal contraceptives and progestins are often the first options because they are accessible, effective, and generally safe. However, their effectiveness can differ among patients, and symptoms frequently return after stopping the treatment. GnRH agonists and antagonists provide stronger suppression of ovarian function and estradiol production. While GnRH agonists are effective, the initial flare-up and low estrogen side effects limit their long-term use. In contrast, GnRH antagonists offer quicker and more controlled suppression of gonadotropins without the flare effect. They also have the added benefit of being taken orally. Despite these advantages, concerns about bone mineral density and long-term safety are still crucial to consider (20). Aromatase inhibitors are another option, especially for difficult cases. They target local estrogen production in endometriotic tissue but their use is limited due to low estrogen side effects and the need for combination therapy (23). Recent advances in molecular research have enhanced our understanding of the complex biology of endometriosis, particularly the roles of inflammation, angiogenesis, and changes in the immune response. This progress has led to new targeted therapies that focus on specific signaling pathways and inflammatory substances (7). Even though these new treatments show promise, they are mostly still experimental and need more clinical testing. Treatment plans work best when tailored to fit the individual needs and symptom levels of each patient. Factors such as patient age, reproductive goals, other health conditions, and tolerance for side effects play important roles in this customization.

Conclusion

Endometriosis is a disease that is chronic and dependent on estrogens and therefore requires long-term and personalized treatment. While existing medications for pain reduction and control of disease activity can effectively treat the symptoms of endometriosis, they are mostly palliative and not curative. Various hormonal treatments such as the combination of oral contraceptives, progestins, GnRH analogs, and GnRH antagonists currently remain the core of the drug therapy for endometriosis with each having certain advantages and disadvantages associated with adverse effects and their duration. Aromatase inhibitors can be used as alternative treatment in certain cases. However, relapse after discontinuing the treatment with drugs remains the issue. Thus, it is important to further develop existing approaches through research to improve their effectiveness. The development of target and gene-based therapies seems promising in terms of targeting pathogenic mechanisms instead of just treating symptoms of endometriosis.

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Digital therapeutic products in community pharmacy practice. Applications and perspectives

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Digital therapeutic products (DTx) are software-based medical devices designed to support the treatment and monitoring of patients with various health conditions. They can be used as stand-alone interventions or as an adjunct to pharmacological therapy and are increasingly applied in outpatient healthcare settings. The aim of this review is to summarize the available scientific evidence on the use of digital therapeutic products in community pharmacy practice, with a focus on reported clinical outcomes, regulatory frameworks, and pharmacists' involvement in their implementation and monitoring. A narrative overview of publications from international scientific databases was conducted, focusing on studies on the application of DTx in chronic diseases, medication adherence, and patient self-management. In addition, regulatory requirements governing the approval and use of digital therapeutic products in different countries were reviewed. The available evidence suggests that digital therapeutic products may improve treatment monitoring and enhance patient engagement. Pharmacists may have a supportive role in informing patients, facilitating access to appropriate digital solutions, and monitoring their use. At the same time, several challenges remain, particularly regarding regulatory clarity, reimbursement, and practical implementation. In conclusion, digital therapeutic products represent an innovative and promising tool with potential applications in community pharmacy practice. Further research and clearly defined implementation frameworks are needed to support their wider adoption.

Introduction

Digital therapeutics (DTx) are evidence-based therapeutic interventions driven by high-quality software programs intended to prevent, manage, or treat medical disorders and diseases [1]. Unlike general wellness or lifestyle applications, DTx products are typically developed as software-based medical devices and are supported by clinical evidence demonstrating their therapeutic value [2]. They may be used independently or in combination with pharmacological therapy and are increasingly recognized as part of modern digital healthcare systems. The rapid expansion of digital health technologies, accelerated further by the COVID-19 pandemic, has increased interest in remote patient monitoring, telehealth, and personalized digital interventions. Within this context, DTx products have emerged as promising tools for improving disease management, medication adherence, and patient self-management, particularly in chronic conditions such as diabetes mellitus, asthma, cardiovascular diseases, insomnia, and mental health disorders [3]. Recent evidence suggests that DTx interventions may improve patient engagement and facilitate continuous monitoring outside traditional healthcare settings [4].

Community pharmacists are among the most accessible healthcare professionals and play an increasingly important role in patient-centered care, chronic disease management, and medication optimization [5]. The integration of digital technologies into pharmacy practice has created new opportunities for pharmacists to support patients through education, monitoring, and digital health counseling. Pharmacists may contribute to the implementation of DTx by assisting patients in selecting appropriate digital therapeutic products, improving adherence to therapy, interpreting digitally collected health data, and collaborating with physicians and other healthcare professionals [6]. Several studies have demonstrated the potential benefits of pharmacist-led digital health interventions in improving medication adherence and clinical outcomes.

Telepharmacy and mobile health interventions, for example, have shown positive effects in diabetes management, hypertension control, and patient self-management. Nevertheless, despite growing interest in DTx, their integration into routine community pharmacy practice remains limited due to challenges related to regulatory approval, reimbursement models, interoperability, digital literacy, and workflow integration [4–6].

In addition, the regulatory landscape for DTx products continues to evolve internationally. In the United States, several DTx products have received authorization through the Food and Drug Administration (FDA), while in Europe, digital therapeutics are increasingly regulated under the Medical Device Regulation (MDR) framework [7]. However, differences between healthcare systems, reimbursement pathways, and implementation standards create uncertainty regarding the broader adoption of DTx in everyday clinical and pharmacy practice. Given the expanding role of digital healthcare and the accessibility of community pharmacists, understanding the current evidence regarding DTx implementation in pharmacy practice is increasingly important. Therefore, the aim of this narrative review is to summarize the available scientific evidence on digital therapeutic products in community pharmacy practice, with particular focus on clinical outcomes, regulatory considerations, and the role of pharmacists in their implementation and monitoring.

Methods

This study was a narrative literature review aimed at summarizing the available scientific evidence on the use of digital therapeutic products (DTx) in community pharmacy practice. The review focused on the role of pharmacists in the implementation and monitoring of DTx, reported clinical outcomes, and the regulatory frameworks governing their approval and use.

A structured literature search was performed using several international scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. Publications published in English between 2019 and 2025 were included to include recent evidence on digital therapeutics and pharmacy practice. The search strategy included combinations of the following keywords: “digital therapeutics”, “digital therapeutic products”, “community pharmacy”, “pharmacists”, “digital health”, “mobile health”, “telepharmacy”, “medication adherence”, “patient monitoring”, and “self-management”. Boolean operators such as “AND” and “OR” were used to refine the search process.

Studies were included if they addressed the application of DTx products in clinical or outpatient settings, evaluated pharmacist involvement in digital health interventions, or reported outcomes related to medication adherence, patient engagement, disease monitoring, or patient self-management. Regulatory and policy publications related to digital therapeutics were also reviewed. Original research articles, clinical studies, systematic reviews, and narrative reviews were considered eligible for inclusion. Publications unrelated to healthcare applications, non-English articles, conference abstracts without accessible full text, and studies focused exclusively on general wellness applications without therapeutic purpose were excluded.

Titles and abstracts identified through the database search were initially screened for relevance, after which potentially eligible full-text articles were reviewed in detail. Relevant information was extracted and organized by type of digital therapeutic intervention, targeted disease or condition, clinical outcomes, pharmacist involvement, implementation barriers, and regulatory considerations.

Due to the heterogeneity of the identified studies, including differences in study design, interventions, and reported outcomes, a qualitative narrative synthesis was performed rather than a quantitative meta-analysis. The findings were analyzed descriptively to identify common themes, current trends, and existing gaps in the literature on the integration of digital therapeutics into community pharmacy practice.

In addition to the scientific literature review, publicly available regulatory documents and guidance materials from the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and relevant European Union Medical Device Regulation (MDR) documentation were reviewed to summarize current regulatory approaches related to the approval and implementation of DTx products.

Results

The reviewed literature demonstrated growing interest in the implementation of digital therapeutic products (DTx) within healthcare systems and outpatient care settings. The analyzed publications showed that DTx interventions are increasingly applied in the management of chronic diseases, mental health disorders, medication adherence, and patient self-management. Several studies reported that digital therapeutics may improve patient engagement, facilitate continuous monitoring, and support personalized healthcare through software-based therapeutic interventions [8–11].

Findings also highlighted the expanding roles of pharmacists and other healthcare professionals in implementing DTx products. Pharmacists may contribute through patient counseling, education, therapy monitoring, and support for the appropriate use of digital health technologies. Digital health interventions involving pharmacists were associated with improved adherence to therapy and enhanced communication between patients and healthcare providers.

At the same time, the reviewed studies identified several important barriers limiting the broader adoption of DTx in routine clinical and pharmacy practice. The most frequently reported challenges included insufficient regulatory harmonization, lack of reimbursement frameworks, interoperability issues, concerns regarding data privacy and cybersecurity, and varying levels of digital literacy among patients and healthcare professionals.

In addition, the literature highlighted the rapidly evolving regulatory landscape surrounding DTx and software-based medical devices. Current regulatory pathways in the United States and Europe increasingly recognize digital therapeutics as evidence-based medical interventions; however, clearer implementation standards and stronger clinical validation remain necessary to support their wider integration into healthcare systems.

Overall, the evidence analyzed suggests that digital therapeutics are a promising and innovative tool for supporting patient-centered care and chronic disease management. Nevertheless, further clinical research, multidisciplinary collaboration, and well-defined regulatory and reimbursement frameworks are needed to facilitate their successful implementation in community pharmacy practice.

A recent study on the current regulatory status of digital therapeutics (DTx) and Software as a Medical Device (SaMD) within the FDA Breakthrough Device Program in the United States and the Korean Innovative Medical Device Program. The authors emphasized that regulatory support programs may accelerate the development and clinical implementation of innovative DTx products, particularly those based on artificial intelligence technologies.

The study also highlighted that the rapid expansion of digital therapeutics requires adaptive regulatory frameworks, stronger collaboration between developers and regulatory authorities, and clearer policies related to clinical validation, safety, and reimbursement. These findings demonstrate the importance of establishing standardized regulatory pathways to support the wider integration of DTx into healthcare and pharmacy practice.

A review published in the European Review for Medical and Pharmacological Sciences emphasized that although digital therapeutics (DTx) represent an innovative approach in digital medicine, their implementation raises important ethical and practical concerns related to data privacy, accessibility, patient autonomy, and clinical decision-making [9]. The authors noted that while DTx may improve personalization of care and support chronic disease management, barriers such as digital literacy, infrastructure limitations, and unequal access to technology may affect their broader implementation in healthcare systems.

The review further highlighted the need for transparent regulatory oversight and multidisciplinary collaboration to ensure the safe and effective integration of DTx into clinical practice. These findings support the importance of establishing clear implementation frameworks and professional guidance for healthcare providers, including community pharmacists, involved in the use and monitoring of digital therapeutic products. Rosa, A. D. (2022, September 30). Ethics of digital therapeutics (DTx). European Review. [9]

A recent review discussed the growing adoption of digital therapeutics (DTx) in healthcare systems and emphasized their potential to improve chronic disease management, patient engagement, and personalized care through evidence-based software interventions. The authors highlighted that DTx products are increasingly being integrated into clinical practice as regulated software-based medical devices, particularly in areas such as diabetes, mental health disorders, and medication adherence [11].

The publication also noted that successful implementation of DTx requires multidisciplinary collaboration between healthcare professionals, including pharmacists, physicians, and digital health developers. In addition, the authors emphasized that reimbursement policies, regulatory harmonization, and digital literacy remain major barriers to the wider adoption of digital therapeutics in routine healthcare practice.

Figure 1 presents the main benefits and challenges associated with the implementation of digital therapeutic products in healthcare and community pharmacy practice. Reported benefits include improved treatment monitoring, increased medication adherence, enhanced patient engagement, and more personalized care. However, several barriers continue to limit the broader adoption of DTx, including regulatory uncertainty, reimbursement limitations, difficulties with workflow integration, and concerns about data privacy and cybersecurity.

Table 1 summarizes representative examples of currently available digital therapeutic products and their potential relevance to community pharmacy practice. The analyzed DTx products are primarily used to manage chronic diseases, mental health disorders, and insomnia, and to support medication adherence, with key functions including behavioral therapy, glucose monitoring, inhaler tracking, and patient support. The findings suggest that community pharmacists may help implement these technologies by providing patient counseling, monitoring treatment adherence, and facilitating access to appropriate digital therapeutic solutions [10].

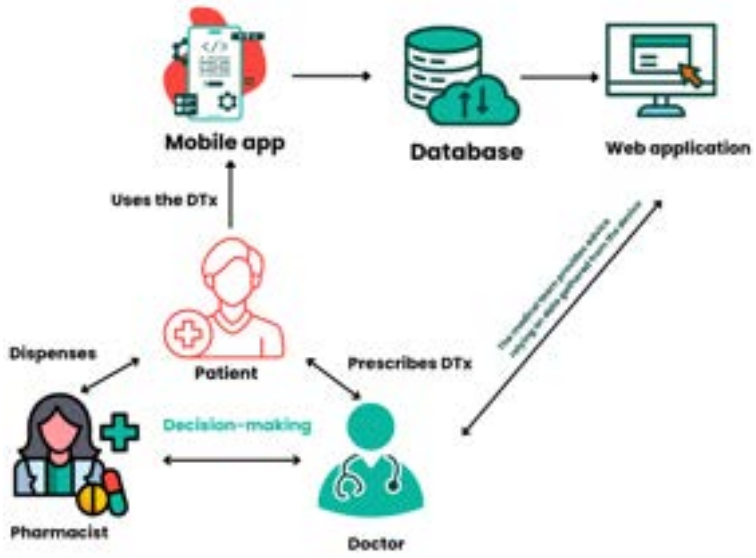

Figure 1. Chronic disease management DTx.

Table 1. Patient-centered decision-making using DTx data

Digital medical device DTx	Indication	Key Function	Community Pharmacy
reSET-O	Opioid dependence	CBT therapy	Counseling support
Somryst	Insomnia	Behavioral therapy	Patient education
BlueStar	Diabetes	Glucose management	Monitoring
Propeller	Asthma/COPD	Inhaler tracking	Adherence support

Discussion

The findings of this review demonstrate the growing importance of digital therapeutic products (DTx) as innovative tools in modern healthcare and their potential integration into community pharmacy practice. The analyzed studies suggest that DTx may support chronic disease management, improve medication adherence, enhance patient engagement, and facilitate remote monitoring through evidence-based software interventions [8–11]. These findings are consistent with the broader digital transformation of healthcare, where personalized and technology-assisted approaches are increasingly implemented to improve clinical outcomes and patient-centered care [12].

Community pharmacists appear to have an important supportive role in the implementation of DTx products [10]. Due to their accessibility and frequent patient contact, pharmacists may contribute to patient education, identify appropriate digital therapeutic solutions, monitor adherence, and interpret digitally collected health information [13–15]. The reviewed literature also suggests that pharmacist-led digital interventions may improve communication between patients and healthcare providers and support more effective self-management of chronic conditions [6].

At the same time, several barriers continue to limit the wider adoption of DTx in routine pharmacy practice. One of the most significant challenges identified in the reviewed studies is the lack of standardized regulatory and reimbursement frameworks. Although regulatory agencies such as the FDA and European authorities increasingly recognize DTx as software-based medical

devices, differences between national healthcare systems and approval pathways create uncertainty regarding implementation and long-term sustainability. In addition, concerns related to data privacy, cybersecurity, interoperability, and integration into existing pharmacy workflows remain important practical limitations [1][8].

Another important consideration is digital literacy among both patients and healthcare professionals. Successful implementation of DTx requires not only technological infrastructure but also sufficient training and confidence in the use of digital health tools. Pharmacists may therefore require additional education and professional training to effectively integrate DTx into pharmaceutical care services [16].

Despite these challenges, the reviewed evidence indicates that digital therapeutics have considerable potential to expand the scope of community pharmacy services and support more individualized patient care [5–12]. Future research should focus on evaluating long-term clinical outcomes, cost-effectiveness, patient acceptance, and the impact of pharmacist involvement in DTx implementation. Furthermore, the development of clear professional guidelines and regulatory standards may facilitate broader integration of digital therapeutics into healthcare systems.

Conclusion

Digital therapeutic products represent an emerging and promising component of digital healthcare with potential applications in community pharmacy practice. The available evidence suggests that DTx may improve patient engagement, medication adherence, disease monitoring, and self-management, particularly in chronic disease care. Community pharmacists may play an important role in supporting the implementation and monitoring of these technologies through patient counseling and digital health support.

However, several challenges remain regarding regulatory harmonization, reimbursement, digital literacy, workflow integration, and data security. Further research and clearly defined implementation frameworks are necessary to support the safe, effective, and sustainable integration of digital therapeutics into routine pharmacy practice and healthcare systems.

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Application of nano spray drying in the formulation of drug-loaded nanoparticles: a review

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Nano spray drying has emerged as an innovative particle engineering platform that overcomes the critical bottlenecks of conventional spray drying – namely, broad particle size distributions, low submicron collection efficiencies, and the thermal degradation of labile compounds. By integrating piezoelectric vibrating mesh atomizers with electrostatic particle precipitators, this continuous, single-step technology enables the predictable fabrication of uniform submicron and nanoscale particles directly from liquid feeds. This review provides a comprehensive overview of nano spray drying in the development of drug-loaded nanoparticles. It delineates the foundational principles of atomization, drying kinetics (modeled via the Péclet number), and electrostatic deposition mechanisms. Furthermore, it analyzes the interplay between critical formulation variables (viscosity, solute concentration, solvent volatility) and operational parameters (inlet temperature, feed rate, gas flow, and mesh aperture size) in governing final nanoparticle attributes such as size distribution, morphology, solid-state configurations, zeta potential, and encapsulation efficiency. The pharmaceutical utility of the platform is highlighted through its diverse applications, including accelerating the dissolution kinetics of poorly water-soluble compounds, customizing matrices for controlled-release architectures, engineering respirable powders for deep pulmonary delivery, and stabilizing thermolabile biologics like proteins and vaccines. Finally, we provide a technical assessment contrasting nano spray drying against alternative methodologies (nanoprecipitation, emulsion-based techniques, lyophilization, and supercritical fluid processes), while discussing current scaling bottlenecks and future integration with artificial intelligence (AI) for digitized process optimization.

Introduction

Nanoparticles have emerged as pivotal drug delivery systems due to their versatile functionality, enhanced bioavailability, and capacity for controlled and targeted release. Approximately 90% of pipeline drug candidates exhibit poor aqueous solubility; nanoscale carriers can significantly increase their effective surface area, thereby accelerating dissolution rates and improving oral bioavailability. Beyond solubility enhancement, nano-delivery systems optimize pharmacokinetic profiles, minimize systemic toxicity, and enable targeted accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect. Consequently, nanotechnology has been extensively investigated across various administration routes – including intravenous, intranasal, oral, and topical – as a cornerstone for next-generation therapeutics [1, 2].

Various conventional methods exist for producing drug-loaded nanoparticles, each presenting distinct advantages and limitations dictated by the drug's physicochemical properties and intended route of administration. Bottom-up techniques like nanoprecipitation offer simple, low-energy preparation but are highly sensitive to processing parameters, which often limits reproducibility, particle size control, and the encapsulation efficiency of hydrophilic molecules. Emulsification-based methods accommodate both hydrophilic and hydrophobic compounds but require organic solvents and stabilizing surfactants that raise toxicity and regulatory concerns, while also suffering from emulsion instability and poor scalability. Top-down approaches, such as high-pressure homogenization and wet milling, are scalable for pharmaceutical nanocrystals but consume substantial energy and exert high

mechanical stress (cavitation, shear forces), which risks degrading thermolabile compounds. Overall, these traditional methodologies frequently suffer from uneven particle size distributions, necessitating additional homogenization steps and limiting precise control over nanoparticle properties [3, 4].

Conventional spray drying is widely utilized in pharmaceutical processing to convert liquid formulations into dry powders for oral and inhalation routes in a single, continuous, and cost-effective step. However, its utility in nanoparticle engineering is severely restricted. Traditional dual-fluid or rotary atomizers generate micrometer-sized droplets that yield particles typically ranging from 1 to 20 m. Furthermore, variations in airflow, temperature, and nozzle dynamics often result in broad particle size distributions and poor batch-to-batch reproducibility. Standard cyclone separators also exhibit low collection efficiencies for submicron particles, which escape into the exhaust stream and drastically reduce product yield. Additionally, the high thermal exposure required for drying can degrade sensitive biomacromolecules like proteins and peptides [5, 6].

To overcome these limitations, nano spray drying has emerged as a precise, innovative particle engineering platform capable of fabricating submicron and nanoscale particles with tailored morphologies. This technology utilizes a vibrating mesh/piezoelectric atomizer to generate highly uniform droplets, paired with an electrostatic particle collector that efficiently captures ultra-fine particles via electrical charge deposition. Operating under relatively mild thermal conditions, nano spray drying preserves the structural integrity of thermolabile compounds, including proteins, peptides, and vaccines. Crucially, the system can process sample volumes as small as a few milliliters, making it an invaluable tool during early-stage formulation development where drug availability is limited [7].

This review provides a comprehensive and critical overview of nano spray drying in drug-loaded nanoparticle development. It delineates the fundamental principles of the technology, analyzes critical process and formulation parameters governing particle characteristics, and summarizes recent applications spanning small molecules and biologicals. Finally, a comparative assessment with conventional techniques is presented alongside an evaluation of current scale-up, optimization, and industrial implementation challenges.

Fundamental principles of nano spray drying

Nano spray drying converts liquid solutions or suspensions into ultra-fine dry powders via a continuous, three-step sequence: atomization, rapid solvent evaporation, and electrostatic particle collection. By integrating these stages, the technology exerts precise control over particle formation, making it a reliable and scalable tool for nanoparticle fabrication. Unlike conventional cyclone-based systems, which fail to capture submicron fractions, this advanced process achieves high-yield recovery of nanoscale matrices under mild thermal conditions [8, 9]. The overall process and its major stages are schematically illustrated in **Figure 1**.

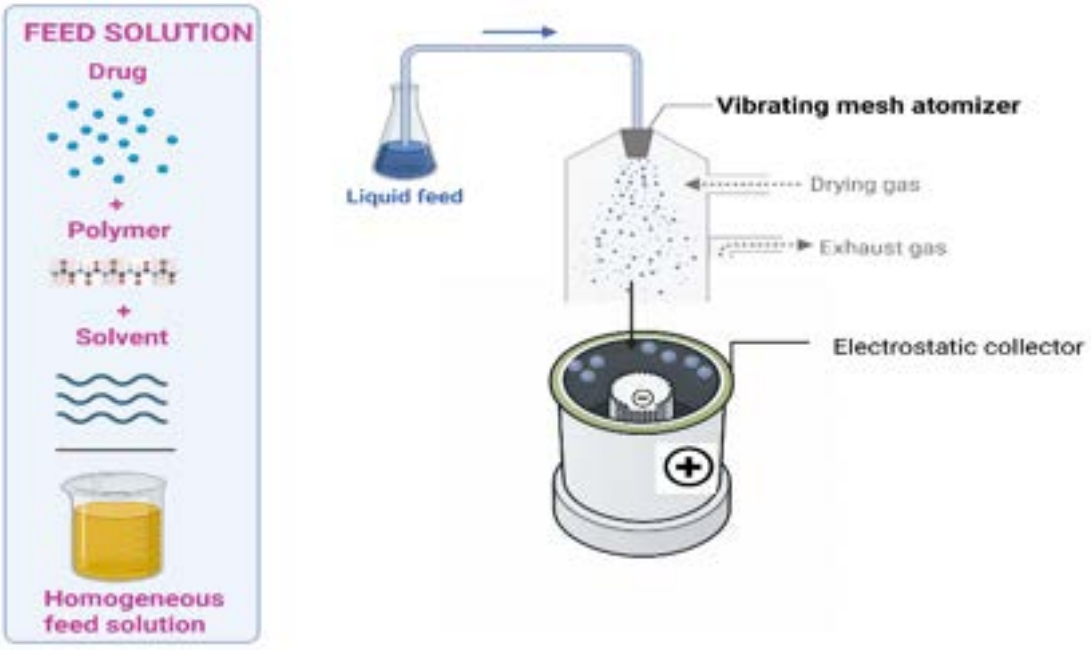

Figure 1. Schematic representation of the nano spray drying process. Created in BioRender, 2026.

Atomization is the critical initial stage that dictates droplet size, homogeneity, and subsequent nanoparticle size distribution. Conventional spray drying employs dual-fluid or rotary nozzles that yield broad droplet distributions highly sensitive to fluid viscosity and atomization energy. In contrast, nano spray drying utilizes a vibrating mesh or piezoelectric membrane atomizer to generate highly uniform droplets. Driven by a piezoelectric actuator, the liquid formulation is driven through a micro-engineered membrane containing micron-sized pores. Droplet diameters are precisely controlled and reproduced by selecting specific membrane mesh sizes, ensuring a narrow particle size distribution and highly consistent batch-to-batch product quality [10].

Following atomization, the generated droplets enter a laminar stream of heated drying gas (typically air or nitrogen), initiating rapid solvent evaporation and solid phase transition. Due to the high surface-area-to-volume ratio of submicron droplets, solvent removal occurs almost instantaneously. This ultra-short thermal exposure protects heat-sensitive biomacromolecules, such as proteins and peptides, from thermal degradation [11].

The internal structure and final morphology of the dried particles are governed by the competitive kinetics between solvent evaporation (droplet shrinkage) and solute diffusion. This balance is quantitatively modeled by the dimensionless Péclet number. Solvent evaporation outpaces solute diffusion, causing solutes to accumulate rapidly at the droplet boundary. This leads to early shell formation and yields hollow, porous, or collapsed particles. Diffusive transport allows solutes to redistribute uniformly toward the core during droplet shrinkage, producing dense, homogeneous solid particles [12, 13].

The final phase involves separating and harvesting the dry nanoparticles from the exhaust gas stream. Standard cyclone separators rely on centrifugal forces, which possess low collection efficiency for submicron particles, resulting in extensive material loss. Nano spray drying overcomes this barrier by

employing an electrostatic precipitator. Particles receive an electrical charge during the process and are subsequently attracted to a cylindrical collection electrode of opposite polarity. The deposition efficiency is regulated by the particle charge, particle size, and the applied electric field intensity. Furthermore, this gentle electrostatic deposition minimizes particle agglomeration and eliminates high mechanical shear stress, preserving the structural integrity and surface morphology of the engineered nanoparticles while maximizing overall product yield [14, 15].

Critical process and formulation parameters

The properties and performance of nanoparticles engineered via nano spray drying are governed by a complex interplay between formulation (solution) properties and operational process parameters. Optimizing these critical variables is mandatory to ensure batch-to-batch reproducibility, high product yield, and desired drug release profiles.

The physicochemical attributes of the feed liquid fundamentally dictate droplet stability, drying kinetics, and final particle architecture. The viscosity of the feed solution directly influences the fluid dynamics across the vibrating mesh. Excessively low-viscosity solutions generate an unstable liquid jet during piezo-actuation, which degrades particle structural stability and uniformity. Higher feed concentrations increase the mass available per droplet, consistently generating larger, denser solid particles. Conversely, dilute solutions promote the formation of smaller, ultra-fine, and often more porous matrices. Since particle solidification depends on solvent evaporation, solvent volatility (boiling point, vapor pressure, and latent heat of vaporization) is a critical formulation variable. High-volatility solvents drive rapid shell formation, leading to hollow or porous structures, whereas low-volatility solvents sustain longer drying windows, producing dense, compact nanoparticle [16, 17].

Process conditions precisely modulate heat and mass transfer within the drying chamber, directly altering particle morphology, size distribution, and yield. The inlet temperature dictates the thermal energy available for solvent evaporation. Elevated inlet temperatures accelerate droplet drying and cause rapid surface crusting, which induces hollow or wrinkled morphologies. However, excessively high temperatures risk the thermal degradation of heat-sensitive active ingredients (e.g., proteins, peptides) or low-glass-transition polymers. Thus, thermal boundaries must be carefully balanced. The gas flow rate determines the droplet residence time and convective heat exchange efficiency. Insufficient gas flow limits moisture removal, leading to incomplete drying and severe particle agglomeration on the chamber walls. The rate at which the liquid formulation is supplied to the atomizer alters the droplet mass balance. Higher feed rates overload the vibrating mesh, generating larger droplets, restricting drying uniformity, and increasing the residual solvent content within the final product. Unique to nano spray drying, the choice of the replaceable vibrating mesh membrane is the primary determinant of initial droplet size. Smaller mesh apertures generate ultra-fine droplets that yield true submicron/nanoscale particles with narrow size distributions, whereas larger apertures shift the distribution toward the micrometer range [18, 19].

The comprehensive relationships mapping these critical parameters to the resulting nanoparticle properties are systematically summarized in **Figure 2**.

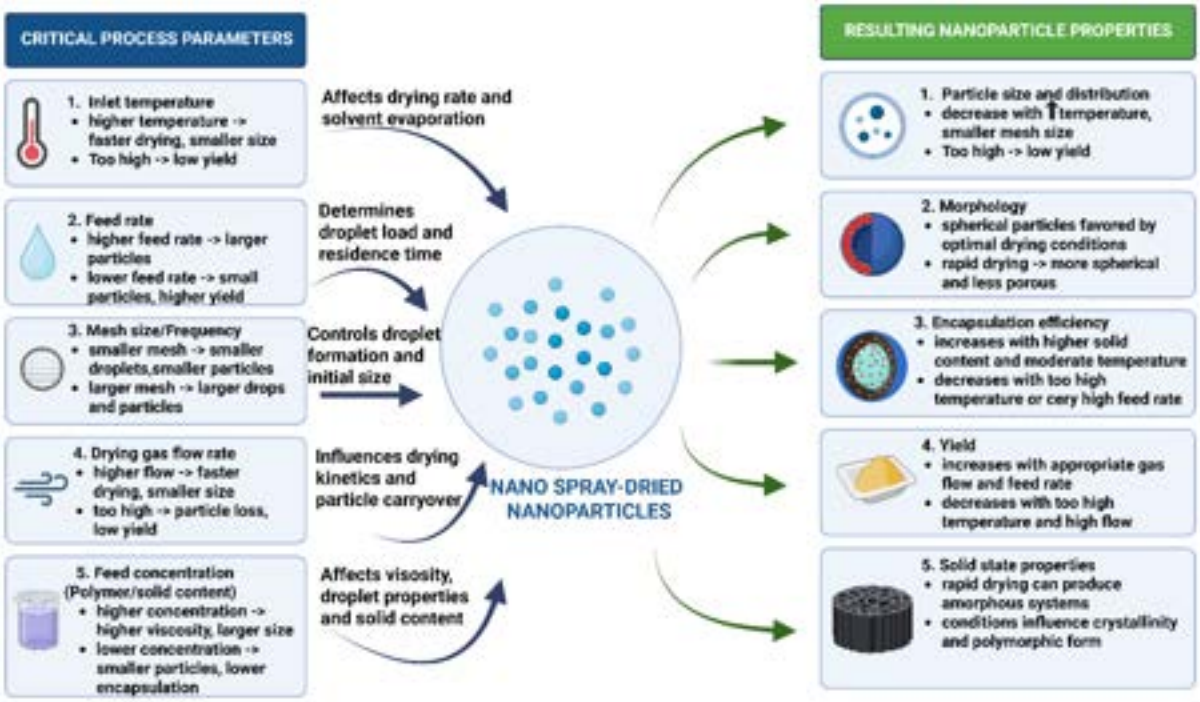

Figure 2. Influence of critical process parameters on the physicochemical properties of nano spray-dried nanoparticles, including particle size, morphology, encapsulation efficiency, yield, and solid-state characteristics, Created in BioRender, 2026.

Physicochemical characterization of engineered nanoparticles

Comprehensive characterization of nano-spray-dried particles is essential to ensure batch uniformity, stability, and therapeutic reproducibility. Particle size and the polydispersity index (PDI) directly modulate dissolution velocity, cellular uptake, and in vivo pharmacokinetics. Nanoscale engineering maximizes specific surface area, accelerating the dissolution rate of poorly water-soluble compounds. Evaluation of these metrics is routinely performed using dynamic light scattering (DLS), laser diffraction, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Lower PDI values denote high monodispersity and superior systemic reproducibility, which are essential for quality control in nano-delivery systems [20].

Nanoparticle morphology governs powder rheology, aerosolization efficiency, and physical stability. While nano-spray-dried matrices are predominantly spherical, their surface topography varies based on composition and drying kinetics. Accelerated solvent evaporation triggers early crust formation at the droplet perimeter, leading to hollow, wrinkled, or high-porosity structures due to internal vapor pressure differentials. Incorporating polymeric or surfactant stabilizers modulates shell elasticity and mitigates aggregation. Although the vibrating mesh atomizer ensures highly uniform droplet precursors, downstream particle aggregation can still occur during collection or storage due to high surface free energy, necessitating rigorous morphological screening [21, 22].

Nano-spray drying frequently promotes the formation of amorphous solid dispersions because ultra-rapid solvent evaporation restricts molecular rearrangement and crystal lattice growth. The higher free energy of the amorphous state significantly enhances the apparent solubility and

dissolution rate of BCS Class II and IV drugs. Solid-state configurations are typically verified via differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). The conversion from a crystalline lattice to a molecularly dispersed amorphous state within the carrier matrix is characterized by the complete disappearance of characteristic drug endothermic melting peaks on DSC thermograms and the absence of sharp Bragg reflections in XRPD patterns. Because the amorphous state is thermodynamically unstable and susceptible to moisture-induced recrystallization during storage, selecting optimal stabilizing polymers or sugars is critical to preserving long-term structural integrity [23, 24].

Zeta potential quantifies particle surface charge and serves as a primary indicator of colloidal stability. Absolute values exceeding generate sufficient electrostatic repulsive forces to prevent particle aggregation in suspension. The surface charge is highly dependent on the ionized functional groups of the matrix polymers, surfactants, and encapsulated cargo. Measured via electrophoretic light scattering, monitoring shifts in -potential over time is an essential metric for detecting surface restructuring, ligand degradation, or colloidal instability during storage [25, 26].

Encapsulation efficiency (EE%) quantifies the carrier's capacity to retain active pharmaceutical ingredients (APIs) during the drying process. EE% is regulated by API-polymer affinity, solute concentrations, and drying rates. Hydrophobic drugs typically exhibit high EE% due to favorable thermodynamic interactions with lipophilic polymeric cores. Conversely, hydrophilic molecules often migrate toward the droplet periphery during rapid evaporation, causing surface accumulation and lower retention. EE% is determined either indirectly by quantifying the unencapsulated drug fraction in the supernatant, or directly by dissolving the harvested nanoparticles, followed by quantification via high-performance liquid chromatography (HPLC) or UV-Vis spectroscopy [27, 28].

Pharmaceutical Applications

The primary application of nano-spray drying is overcoming the formulation barriers associated with poorly water-soluble APIs by generating amorphous nanostructures with high surface areas. This strategy is highly effective in cancer chemotherapy, where critical therapeutic agents – such as paclitaxel, curcumin, docetaxel, and tamoxifen – suffer from poor aqueous solubility and low oral bioavailability. Encapsulating these agents within polymeric, lipidic, or proteinaceous matrices optimizes drug stability, passive tumor targeting via the enhanced permeability and retention (EPR) effect, and cellular internalization, while reducing systemic toxicities [29, 30].

Spray-dried curcumin-lecithin complexes display significantly superior solubility, improved thermal and gastrointestinal stability, and enhanced oral bioavailability compared to conventional crystalline forms. Nano-spray-dried paclitaxel nanoparticles engineered for inhalation demonstrate optimized aerodynamic diameters, deep pulmonary deposition, extended retention within the lung parenchyma, and augmented local tissue distribution [31, 32].

Nano-spray drying serves as a precise platform for engineering sustained-release matrices by enabling the strategic selection of biocompatible polymers and excipients to modulate drug diffusion, polymer erosion, and release kinetics. Biodegradable polymers – such as poly(lactic-co-glycolic acid) (PLGA), chitosan, alginate, and Eudragit® derivatives – are widely integrated to

optimize pharmacokinetic profiles and sustain localized delivery [33].

The underlying release kinetics are dictated by formulation and processing variables, including particle size, polymer molecular weight, internal morphology, and the spatial distribution of the drug within the matrix. Non-porous, dense nanoparticles lengthen the solvent diffusion pathway, yielding prolonged release profiles, whereas porous architectures facilitate rapid medium penetration and accelerate dissolution. Ultimately, encapsulating therapeutics (e.g., chemotherapeutics, antibiotics, peptides) within these solid-state polymeric dispersion networks shields labile molecules from premature degradation, minimizes plasma concentration fluctuations, reduces dosing frequencies, and enhances patient compliance [34, 35].

Conclusion

Nano spray drying represents a high-precision particle engineering platform that successfully overcomes the limitations of conventional spray drying. By incorporating piezoelectric vibrating mesh atomizers and electrostatic precipitators, this continuous, single-step technology enables the fabrication of highly monodisperse submicron and nanoscale particles under mild thermal conditions.

Physicochemically, the platform excels at producing amorphous solid dispersions that accelerate the dissolution kinetics and oral bioavailability of poorly water-soluble compounds. Furthermore, it allows for the precise modulation of particle density and aerodynamic diameters to optimize pulmonary drug delivery, while safeguarding the structural integrity of thermolabile biomacromolecules such as proteins and vaccines.

Despite these advantages, widespread industrial implementation remains restricted by limited volumetric scale-up, significant material wall losses due to high particle surface energy, and substantial capital costs associated with proprietary components. To bridge the gap between benchtop optimization and commercial manufacturing, future advancements must focus on multi-orifice nozzle scaling, inline Process Analytical Technology, and the integration of artificial intelligence predictive modeling. Ultimately, these innovations will solidify nano spray drying as a cornerstone for next-generation advanced drug delivery systems and personalized medicine.

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IV. Therapeutic Session

Cardiac involvement in sarcoidosis

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Introduction: Sarcoidosis is a multi-system granulomatous inflammatory disorder with unknown etiology. Although the clinical course is unpredictable, the manifestations in the heart range from asymptomatic disease to heart failure, arrhythmias, and sudden cardiac death. **Aim:** To present a literature review on the clinical characteristics, diagnostic difficulties, prognosis, and treatment of cardiac sarcoidosis. **Materials and methods:** A review of published literature using the PubMed database. **Results and discussion:** Diagnosis is challenging because sarcoidosis can affect many organs, is non-specific, and overlaps with other cardiomyopathies. Conventional diagnostic methods are insufficient. The invasive nature of endomyocardial biopsy restricts its routine application despite its high specificity. Cardiac magnetic resonance imaging and positron emission tomography play a key role in detecting myocardial inflammation and fibrosis. Cardiac sarcoidosis has a poor prognosis, particularly in patients with reduced left ventricular systolic function, which is the strongest predictor of adverse outcomes. Treatment is largely empirical and focuses on immunosuppression and management of cardiac complications. **Conclusion:** Cardiac sarcoidosis is associated with increased mortality and variable clinical presentation. Early diagnosis and timely treatment may improve the clinical course and outcome.

Introduction

Sarcoidosis is a multisystem inflammatory disease characterized by the formation of non-caseating granulomas in affected organs. Although its exact etiology remains unclear, current evidence suggests that the disease results from an exaggerated immune response to infectious or environmental antigens in genetically predisposed individuals. This immune dysregulation leads to activation of macrophages and CD4+ T lymphocytes, followed by the release of pro-inflammatory cytokines and mediators that promote granuloma formation and tissue fibrosis (1). The incidence and prevalence of sarcoidosis vary significantly worldwide, with the highest rates reported among African Americans and Northern European populations. Scandinavian countries report incidence rates of approximately 11–15 cases per 100,000 people annually, while the incidence in the United States ranges between 8 and 11 cases per 100,000. Lower rates are observed in East Asian countries such as Japan and South Korea. African American women are among the most affected groups and often experience more severe disease and higher mortality (1). Sarcoidosis is most commonly diagnosed between 25 and 45 years of age, with a second peak in women over 50 years, particularly in European and Japanese populations (2).

The disease can involve virtually any organ system; however, the lungs and intrathoracic lymph nodes are affected in approximately 90–95% of cases, commonly presenting with pulmonary infiltrates and bilateral hilar lymphadenopathy. Its clinical presentation is highly heterogeneous, ranging from asymptomatic disease with spontaneous remission to chronic progressive forms associated with significant morbidity. Among the extrapulmonary manifestations, cardiac involvement represents one of the most severe and potentially life-threatening complications of sarcoidosis. Clinically manifest

cardiac sarcoidosis (CS) occurs in approximately 5–10% of patients with systemic disease, although autopsy and advanced imaging studies suggest that subclinical cardiac involvement may be present in up to 25% of cases. Granulomatous infiltration of the myocardium can lead to inflammation, fibrosis, and scar formation, predisposing patients to conduction abnormalities, ventricular arrhythmias, heart failure, and sudden cardiac death. In many patients, cardiac sarcoidosis may even represent the first or isolated manifestation of the disease (1).

Early recognition of cardiac involvement is essential, as timely initiation of immunosuppressive therapy and appropriate management of arrhythmias or heart failure may improve outcomes and reduce complications. Despite the historically poor prognosis associated with cardiac sarcoidosis, recent studies report significantly improved survival rates with contemporary diagnostic and therapeutic strategies, with 5-year survival exceeding 90% in several modern cohorts (3, 4).

Aim

This review aims to summarize the current understanding of cardiac involvement in sarcoidosis, with a focus on its clinical presentation, diagnostic methods, imaging modalities, and the challenges associated with accurate diagnosis and risk stratification.

Materials and methods

A comprehensive literature review was conducted using the international databases PubMed, Scopus, and Web of Science.

Results and discussion

Pathophysiology: Sarcoidosis is a multisystem disease of uncertain aetiology distinguished by non-caseating granulomas in numerous organs. It affects the lungs and intrathoracic lymph nodes in approximately 90% of the cases. However, any organ can be affected, which is responsible for it's highly variable clinical appearance. In the case of cardiac involvement, it is clinically significant as it is associated with arrhythmias, high-grade blocks, heart failure, and sudden cardiac death. The pathogenesis of CS involves granulomatous inflammation in the myocardium, endocardium, pericardium, as well as the conduction system, resulting in myocardial injury and fibrosis (5).

The main pathogenic hallmark of CS is the production of non-caseating granulomas, which are composed of macrophage, epithelioid cell, and T lymphocyte aggregates. The precise mechanism of sarcoidosis has not yet been fully understood. However, it is largely regarded to be an immunologic response against a new antigen in a genetically predisposed individual. The immune cascade commences when antigen-presenting cells (e.g. Macrophages, Dendritic cells) present antigens to naïve CD4+ T-helper cells, which promote differentiation into Th1 and Th17 cells. These T cells release proinflammatory cytokines such as IL-2, IFN-gamma, TNF-alpha, and IL-12, which stimulate activation of macrophages and lymphocytes to form granulomas. Macrophages differentiate into and may fuse into multinucleated giant cells, leading to the formation of the core of granulomatous lesions. As time passes, a Th1 to Th2 dominance shift may occur in chronic sarcoidosis, in unison with the secretion of IL-4, IL-10, and TGF-beta, leading to activation of fibroblasts and the subsequent deposition of extracellular matrix, which ultimately contributed to tissue fibrosis. Inflammation is sustained through T cell proliferation, which occurs due to high concentrations of IL-6 found in early disease (6).

In recent years, new technologies have been found to screen the human genome for

any genetic variations associated with risk of developing the disease, establish their role in the pathogenesis of the disease, as well as to develop therapeutic targets. In CS, GWASs have revealed a strong link to the major histocompatibility complex (MHC), a key component of antigen presentation to T lymphocytes. The MHC allele HLA-DRB1*1101 considerably increased the risk of sarcoidosis in both black and white populations. Other impacted HLA alleles include HLA-DRB1*14, 15, and HLA-DQB1*0601, which are related with chronic disease in general, with HLA-DQB1*0601 being specifically associated with susceptibility to cardiac sarcoidosis in a Japanese research. However, the HLA-DRB1 allele characterisation in 87 Swedish cardiac sarcoidosis patients revealed no differences from non-cardiac sarcoidosis patients. A combination of HLA-DRB1*04/15 was discovered as a risk factor for extrapulmonary involvement in sarcoidosis, and HLA-DRB1*03 and DQB1*0201 are seen more commonly in patients with Löfgren's syndrome (7).

In CS, the myocardium is typically engaged with the endocardium or epicardium as a disease progression. Granulomas are commonly observed in the basal interventricular septum, left ventricular free wall, right ventricle, and atria. As the condition progresses, the granulomatous inflammation triggers a repair response that results in scarring, as opposed to the scarring seen in myocardial infarction, which is patchy rather than maximal in the subendocardial region or transmural. While the distribution of granulomas in the interventricular septum causes conduction blocks that may respond to steroid therapy, patchy myocardial scarring creates a nest for ventricular tachyarrhythmias, localised aneurysms, and systolic or diastolic LV dysfunction (1).

Clinical Manifestations: The clinical presentation of cardiac sarcoidosis is extremely diverse, ranging from asymptomatic disease to acute heart failure or sudden cardiac death. Symptoms are primarily associated with conduction abnormalities, arrhythmias, and progressive myocardial dysfunction. In certain circumstances, cardiac involvement may be the first manifestation of systemic sarcoidosis. The clinical symptoms of CS vary depending on the disease's location, extent, and activity. The main symptoms are conduction abnormalities, ventricular arrhythmias (including sudden death), and heart failure. These patients are typically very symptomatic, with symptom complexity varying depending on presentation. Furthermore, cardiac symptoms usually dominate extracardiac symptoms, as patients typically have only low-grade pulmonary and no other organ involvement. Indeed, the majority of patients with clinically evident CS have limited extracardiac illness, with up to one-third having isolated CS. Patients with clinically silent CS may have nonspecific chest pain, dyspnoea, and fatigue, which are typically caused by extracardiac illness (8).

One of the most prevalent signs of cardiac sarcoidosis is conduction abnormalities. Conduction abnormalities can frequently be the first sign of the condition, which affects 23–30% of people with CS. It can range from first degree heart block to total heart block. The development of granulomas or scar tissue at the basal septum or close to the nodal artery results in ischaemic damage to the conduction route, which causes conduction problems. Bradyarrhythmias can cause patients to experience dizziness, presyncope, syncope, or exhaustion. A recent study reported that a patient had exertional syncope initially from worsening infranodal disease that became evident even at rest later (9).

Another important clinical characteristic of CS is ventricular arrhythmias, which are a major contributor to morbidity and mortality. The predominant substrate for ventricular tachycardia (VT) in patients with CS is believed to be scar secondary to inflammatory damage from sarcoid granulomas. The pattern and location of involvement vary widely; any segment of the left or right ventricle and any layer of the myocardium may be affected. Within the left ventricle, scar tends to be patchy, with the septum being the most common site of involvement. Within the RV, scarring is usually confluent, and all parts appear to be afflicted equally. Isolated RV involvement is uncommon, implying that RV involvement occurs following left ventricular involvement and may indicate a more advanced stage of disease (10).

Manifest heart failure is not rare, and severe granulomas and myocardial scarring can result in end-stage HF. The most common form of heart failure in cardiac sarcoidosis is dilated cardiomyopathy with left ventricular systolic dysfunction (11). Left ventricular (LV) systolic failure, HF with preserved ejection fraction, or right ventricular failure secondary to pulmonary disease are the main manifestations of cardiomyopathy in sarcoidosis. A recent study suggested that 25% to 75% of cardiac deaths in patients with CS are due to progressive heart failure (12).

The clinical course of cardiac sarcoidosis is often unpredictable. Clinically silent CS may occur in 20% to 25% of patients with systemic sarcoidosis, requiring detection only through screening imaging, such as PET/CMR, rather than symptoms (5). In contrast, patients may display signs of rapidly progressive arrhythmias or advanced heart failure. Due to the potentially fatal consequences associated with CS, early detection and diagnosis are critical to improving clinical outcomes (8).

Diagnostic methods and challenges in cardiac sarcoidosis: The diagnosis of cardiac sarcoidosis is challenging because of its heterogeneous presentation, patchy myocardial involvement, and lack of a single definitive diagnostic test. Patients may present with conduction abnormalities, ventricular arrhythmias, heart failure, or incidental imaging findings, while some remain asymptomatic. Therefore, diagnosis relies on a multimodality approach including electrocardiography (ECG), echocardiography, cardiac magnetic resonance (CMR), fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT), and endomyocardial biopsy (EMB), (13).

ECG is often the first diagnostic test but has limited sensitivity. Common abnormalities include atrioventricular block, especially advanced AV block, right bundle branch block, fragmented QRS complexes, ventricular tachycardia, premature ventricular contractions, and atrial arrhythmias. Holter monitoring increases diagnostic sensitivity by detecting intermittent arrhythmias and contributes to risk stratification (13, 14).

Echocardiography is the first-line imaging modality due to its accessibility, although its sensitivity is relatively low in early disease. Findings may include regional wall motion abnormalities, basal interventricular septal thinning, ventricular dysfunction, wall thickening from oedema, or features mimicking dilated or arrhythmogenic cardiomyopathy. Speckle-tracking echocardiography and reduced global longitudinal strain may help detect early myocardial involvement (15).

CMR has become central in CS evaluation because of its excellent tissue characterization and high sensitivity for myocardial fibrosis. Late gadolinium enhancement (LGE), typically patchy and involving the basal septum or lateral wall, is highly

suggestive of CS. T2-weighted imaging and parametric mapping may additionally identify myocardial oedema and active inflammation. CMR also has high negative predictive value and helps exclude alternative cardiomyopathies, although findings may overlap with myocarditis (15, 16).

FDG-PET/CT complements CMR by detecting active myocardial inflammation through increased glucose uptake. It is useful for identifying extracardiac sarcoidosis, guiding biopsy sites, monitoring treatment response, and assessing prognosis. However, physiological myocardial uptake may reduce specificity and requires strict patient preparation (17).

EMB remains the histological gold standard because it can demonstrate non-caseating granulomas within the myocardium. However, its sensitivity is limited due to the patchy distribution of lesions and sampling error. Imaging-guided biopsy may improve diagnostic yield, but EMB remains invasive and is often reserved for diagnostically uncertain cases (13, 18).

Overall, no single diagnostic modality is sufficient for diagnosing CS. Integration of clinical findings, rhythm assessment, multimodality imaging, and histological data is essential for accurate diagnosis and risk stratification.

Management

Immunosuppressive therapy: Corticosteroids remain the foundation of therapy for active inflammation of the myocardium in CS. High doses of prednisone (0.5–1 mg/kg/day) is given to control the inflammation then the dose is tapered down to a maintenance regime based on symptom monitoring and imaging (19). The goal of the therapy is to suppress the inflammation and prevent the progression to fibrosis and to maintain cardiac function. Due to increasing adverse effects of long-term corticosteroid use, other drugs can be used to maintain disease control. Methotrexate is the most common used agent as well as azathioprine and mycophenolate mofetil. Biological agents including tumour necrosis factor-alpha inhibitors are reserved for refractory disease cases (20).

Arrhythmia management and device therapy: Conduction abnormalities are present in 65% of patients with CS upon presentation, with complete heart block being the most common abnormality at 48%, followed by first-degree atrioventricular block at 44% (21). Thus, permanent pacemaker implantation is indicated for symptomatic bradyarrhythmias. Implantable cardioverter defibrillators (ICD) play a crucial role in prevention of sudden cardiac death. It is indicated for secondary prophylaxis in patients with prior cardiac arrest or sustained ventricular tachycardia. Primary prophylaxis is indicated in those with LVEF $\leq$ 35% or in patients with extensive myocardial scarring seen on cardiac magnetic resonance imaging. Antiarrhythmic medications that may be prescribed include amiodarone, sotalol, and mexiletine for refractory ventricular tachycardia (21).

Heart failure management: Guideline-directed medical therapy for heart failure with reduced ejection fraction should be administered according to standard protocols, which includes beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor antagonists. For patients with advanced heart failure refractory to medical therapy, mechanical circulatory support or cardiac transplantation may also be considered (22).

Role of advanced imaging in guiding management: Cardiac magnetic resonance (CMR) and ^{18}F-fluorodeoxyglucose positron

emission tomography (FDG-PET) is essential in monitoring therapeutic response. FDG-PET is the method of choice for identifying active inflammation and guiding immunosuppressive treatment, while CMR is better suited for assessing cardiac morphology, function, and extent of fibrosis. Hybrid FDG-PET/CMR imaging is a comprehensive approach and is increasingly used in clinics (23).

Prognosis: Outcomes in CS are non-specific and depend on multiple factors, including ventricular function, progression of fibrosis, conduction system disease severity, and when treatment was initiated (13). The prognostic significance of **isolated cardiac sarcoidosis (ICS)** versus systemic disease with cardiac involvement has been an area of active investigation. A prospective study of 198 CS patients found that the prevalence of ICS was 7% using Japanese Circulation Society criteria versus 29% using a multidisciplinary diagnosis criterion. Despite this, patients with ICS had similar rates of major cardiovascular outcomes compared to those with systemic sarcoidosis, including progression of atrioventricular block, worsening ejection fraction ≥ 10%, heart transplant, and death. The study concluded that isolated cardiac sarcoidosis was not an independent predictor of death, heart transplant, or clinical worsening. The prognosis of both forms of disease is similar (24). In contrast to the above study, in a 2021 cohort, 67% of ICS patients experienced the combined outcome of cardiac death, heart failure hospitalization, or fatal ventricular arrhythmia over 31 months of follow-up, compared with 38% of patients with systemic disease, suggesting that isolated cardiac involvement may follow an aggressive course. However, it must be noted that due to heterogeneity of event rates, this comparison of prognosis remains debated (25).

LVEF improvement is noted to be a powerful prognostic marker in cardiac sarcoidosis. In the ILLUMINATE-CS registry in Japan, patients were grouped by LVEF improvement: least improved (≤ 0%), intermediate (0–9%), and most improved (> 9%). Based on the groupings the mortality rates were progressively higher in the least improved and intermediate groups compared to the most improved (26). **Fibrosis burden** detected on CMR, particularly late gadolinium enhancement involving the basal interventricular septum and left ventricular free wall, strongly correlates with arrhythmic risk and sudden cardiac death. The extent of fibrosis is a major factor of both systolic dysfunction and arrhythmias (13). A comprehensive review of CS research priorities emphasises the need for randomised controlled trials evaluating immunosuppressive regimens, validation of diagnostic criteria, clarification of isolated CS natural history, and the ability to group patients based on disease morphology. Additionally, understanding the mechanisms underlying variable disease progression and identifying predictors of treatment response require development.

Biomarkers and genetics: Current diagnostic approaches for CS, including endomyocardial biopsy, FDG-PET and CMR, are restricted by high costs, and invasiveness. These challenges, together with the disproportionate contribution of cardiac involvement to sarcoidosis-related mortality, highlight the need for innovative and widely accessible diagnostic strategies. Emerging serum biomarkers and genomic studies hold promise for transforming the diagnostic landscape. Biomarkers may provide accessible, cost-effective tools to complement established diagnostic methods, while genetic insights could identify individuals at higher risk for cardiac involvement and group these patients based on disease phenotype.

Specific HLA class II alleles, particularly HLA-DQB1*0601, have been associated with increased CS risk, though further validation is needed (27).

Therapeutic advances: The therapeutic landscape for CS is evolving, with recent studying suggesting the role of alternative immunosuppressants and biologic agents in improving disease control while minimising the long-term toxicity of corticosteroids. Prospective randomized controlled trials are urgently needed to establish evidence-based treatment protocols, as current management relies heavily on observational data (28).

Multidisciplinary care: There has been a shift toward using a multidisciplinary team approach for CS patients. Given the complexity of diagnosis and management, specialized centres with multidisciplinary sarcoidosis teams are increasingly recognized as essential for optimizing outcomes. This approach integrates cardiology, rheumatology, pulmonology, radiology, electrophysiology expertise, and palliative care specialists (28).

Conclusion

Cardiac sarcoidosis is a serious manifestation of sarcoidosis associated with conduction abnormalities, ventricular arrhythmias, heart failure, and sudden cardiac death. Its diagnosis remains challenging because of its heterogeneous and often subclinical presentation. Advances in multimodality imaging, particularly CMR and FDG-PET, have improved early detection and risk stratification, while immunosuppressive therapy and device-based treatment remain essential for management.

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Biological therapy in severe bronchial asthma

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Introduction: Asthma is a heterogeneous chronic disease of the airways with diverse pathogenetic mechanisms, characterized by chronic bronchial inflammation and variable symptoms such as dyspnea, cough, chest tightness, and wheezing. Standard treatment includes reliever and controller medications. In severe asthma that remains symptomatic with frequent exacerbations despite high doses of inhaled corticosteroids (ICS) and long-acting β2-agonists (LABA), biological therapy is applied. This therapy involves monoclonal antibodies that selectively block key mediators of Th2-high eosinophilic inflammation. **Objective:** To investigate the main characteristics of the different pharmacological agents used in so-called biological therapy and their role across the various endotypes of bronchial asthma. To analyze data from pivotal clinical trials demonstrating their clinical efficacy and safety, as well as their potential to achieve complete clinical remission of the disease. **Results and discussion:** Biological agents for bronchial asthma have provided additional therapeutic options for patients who remain inadequately controlled despite optimal controller therapy. They lead to improved quality of life through better symptom control, reduction in the frequency and severity of exacerbations, and decreased need for oral corticosteroid therapy. Treatment success requires a personalized approach, with appropriate patient selection according to the specific asthma endotype and the respective biological agent. Monitoring should include assessment of Th2 inflammatory biomarkers, including blood eosinophil count, serum IgE levels, and fractional exhaled nitric oxide (FeNO). **Conclusion:** Biological therapy is a key element in the personalized treatment of severe bronchial asthma, leading to a lasting and effective impact on the condition and significantly improving disease control.
Keywords: *biological therapy, severe asthma, Th2 inflammation*

Introduction

Bronchial asthma is a chronic inflammatory airway disorder characterized by variable airflow obstruction, airway hyperresponsiveness (AHR) and intermittent symptoms of shortness of breath, cough and wheezing. Despite significant progress in inhalation therapy, the disease remains difficult to control in some patients. This subgroup is defined as severe asthma and represents a significant clinical challenge. Severe asthma is estimated to affect 5% to 10% of individuals with asthma although some studies report a prevalence upwards of 38% globally[1,2].

According to GINA 2026, *severe bronchial asthma is defined as asthma that remains uncontrolled despite optimized treatment with high-dose inhaled corticosteroids (ICS) combined with a second controller medication, most commonly a long-acting β2-agonist (LABA), as well as despite adequate management of contributing factors and comorbidities, or asthma that worsens when attempts are made to reduce high-intensity therapy.* GINA emphasizes that before a patient is classified as having *"severe asthma"*, factors such as incorrect inhaler technique, poor adherence, ongoing exposure to tobacco smoke or allergens, untreated comorbidities, and the possibility of an incorrect diagnosis must be carefully assessed and excluded. GINA 2026 further highlights that a substantial proportion of patients with presumed severe asthma actually have difficult-to-treat asthma rather than true severe asthma.

At the present time, biologics are considered for different phenotypes of asthma, but most often for patients with eosinophilic asthma[3]. Eosinophilia is identified among other clinical biomarkers, according to locally available healthcare resources. Although a universally accepted definition of T2-high asthma has not yet been established, this phenotype is commonly characterized by biomarkers such as an absolute eosinophil count (AEC) ≥ 300 cells/μL, FeNO ≥ 25 ppb, and/or sputum eosinophils between 1% and 3%[4]. Asthma that is predominantly triggered by allergens (allergen-induced

asthma) is defined as allergic asthma. Patients who do not exhibit these features are considered to be T2 low. It is estimated that about 50% or more of all patients with severe asthma exhibit a T2 high endotype, whereas T2 low asthma may make up considerably less of the overall asthma population of around 10% to 30%[5,6]. Each of the biologic agents has a slightly different mechanism of action but similarly target different cytokines or receptors involved in the T2 inflammatory pathway as shown in Fig. 1.

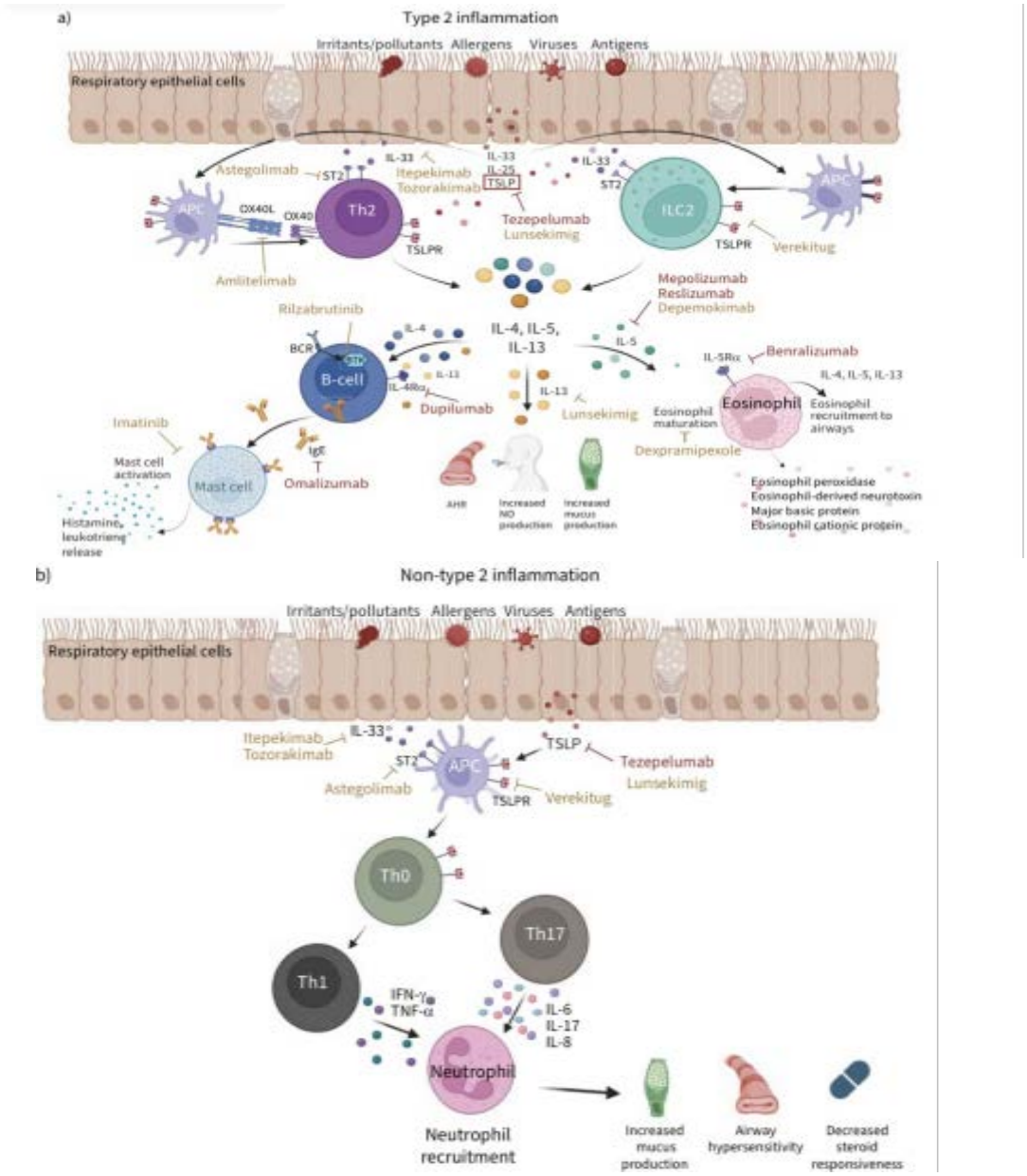

Figure 1. Pathways and key mediators involved in asthma. External stimuli interact at the epithelial level resulting in production of epithelial cytokines (IL-25, IL-33 and TSLP) that can activate both a) type 2 and b) non-type 2 pathways. Type 2 inflammation involves the T-helper 2 cell (Th2) and ILC2 cytokines IL-4, IL-5 and IL-13, which play a role in allergic and eosinophilic asthma as well as nitric oxide (NO) production, mucus production and AHR. Non-type 2 pathways can result in the activation of Th1 and Th17 mediated neutrophilic inflammation. A variety of therapies have been developed that target many components of these pathways (red font) and many other therapies are currently in development. APC: antigen presenting cell; BCR: B-cell receptor; BTK: Bruton's tyrosine kinase; IL-4Rα: IL-4 receptor alpha subunit; ST2: suppression of tumourigenicity 2 receptor; Th0: naive T-cell; TSLPR: TSLP receptor. Figure created with BioRender.com.

The role of phenotyping and endotyping in biologic therapy

To fully appreciate optimal use of biologics, one must have a good understanding of asthma pathophysiology (Fig. 1). A wide range of airborne triggers, including allergens (e.g. dust mites, pollen), pollutants, viruses, tobacco smoke and other irritants, can initiate a cascade of inflammatory responses in the airways. This cascade begins with bronchial epithelial cells secreting cytokines known as alarmins, such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). These alarmins activate antigen-presenting cells (APCs), such as DCs, upregulating OX40 ligand and leading to CD4+T-cell differentiation into T-helper cells (Th1, Th2, Th17 and Th22). TSLP also acts on T-cells, B-cells, Type 2 Innate Lymphoid Cells(ILC2) cells, mast cells, basophils and eosinophils. ILC2 are also triggered by IL-33 via their ST2 receptor[7,8]. Type 2 inflammation involves the T-helper 2 cell (Th2) and ILC2 cytokines IL-4, IL-5 and IL-13, which play a role in allergic and eosinophilic asthma as well as nitric oxide (NO) production, mucus production and AHR. Non-type 2 pathways can result in the activation of Th1 and Th17 mediated neutrophilic inflammation. A variety of therapies have been developed that target many components of these pathways (red font) and many other therapies are currently in development. In up to 80% of patients with severe asthma, the cytokines IL-4, IL-5 and IL-13, secreted from Th2 and ILC2 cells, drive a process termed type 2 inflammation. IL-4 activates B-cells, inducing a class switch to IgE, leading to mast cells degranulation in the presence of aeroallergens and releasing histamines, leukotrienes and prostaglandin D2[9]. Prostaglandin D2 further promotes type 2 inflammation[10]. IgE also activates eosinophils and macrophages via high-affinity IgE receptor (FcεRI), amplifying inflammation[9]. IL-5 drives eosinophil maturation, differentiation, recruitment, activation and survival[11]. Eosinophils release granules contributing to airway inflammation, fibrosis and smooth muscle contraction. IL-13 contributes to AHR, increased nitric oxide production, mucus production and goblet cell metaplasia. While most patients have multiple active type 2 pathways, some patients have only one dominant pathway. Non-type 2 inflammation involves Th1 and Th17 cells, mediated by cytokines such as IFN-γ, TNF-α, IL-6, IL-8 and IL-17, promoting neutrophilic inflammation. Antigen-Presenting Cells(APCs) present antigens to Th0 cells, which differentiate into Th1 or Th17 cells. Neutrophils increase mucus production and airway hyperreactivity and respond poorly to corticosteroids when compared with eosinophils. IL-17 also signals via IL-17A expressed in airway smooth muscle cells[8,12].

Understanding the type of asthma a patient has is essential. Since exposures and pathophysiology are dynam ic, phenotyping helps determine whether a patient has type 2 allergic, type 2 eosinophilic or non-type 2 neutrophilic asthma, or a mixture. In patients with severe asthma, it is crucial to measure biomarkers, such as blood or airway (via sputum or bronchoalveolar lavage) eosinophils, serum IgE and FeNO, to accurately identify endotypes[13]. Elevation in any of these biomarkers categorises asthma as being type 2. The absence of elevated biomarkers, excluding the use of systemic corticosteroids or biologics, indicates non-type 2 asthma. For type 2 inflammation, biomarkers help guide therapy selection. Higher blood eosinophil count (e.g. ≥ 300 cells·μL−1) or FeNO (e.g. > 25 ppb) predicts severe asthma exacerbations and poor control compared to lower counts[8]. IL-5 is a primary target for eosinophil-dominant cases,

IL-4 for IgE-dominant cases and IL-13 for cases with elevated FeNO. Cases with multiple elevated biomarkers without a clear dominant pathway are broadly type 2 asthma.

Type 2 asthma is often associated with comorbidities such as eosinophilic oesophagitis (EoE), allergic bronchopulmonary aspergillosis, eosinophilic granulomatosis with polyangiitis (EGPA), chronic sinusitis, nasal polyps and atopic dermatitis/eczema. These comorbidities may indicate allergic or eosinophilic inflammation and are often treated with the same biologics that are used for asthma. Selecting the most appropriate biologic therapy should involve evaluating the patient's asthma phenotype, endotype and comorbidities, to optimise outcomes.

Biologic therapies according to GINA 2026[14]

The main biologic therapies according to GINA 2026 include anti-IgE, anti-IL5/IL5R, anti-IL4Rα, and anti-TSLP therapies.

Omalizumab is the first anti-IgE biologic therapy and is intended for patients with severe allergic asthma, elevated IgE levels, and confirmed allergic sensitization. GINA 2026 notes that predictors of a good therapeutic response to *omalizumab* include elevated FeNO levels, eosinophilia, an allergic phenotype, and early-onset asthma. The principal therapeutic effects include reduction in exacerbations, decreased need for systemic corticosteroids, and improvement in quality of life.

Anti-IL5 and anti-IL5R therapies include *mepolizumab, benralizumab, reslizumab*, and *depemokimab*. These biologics target eosinophilic inflammation by blocking the IL-5 pathway or by directly depleting eosinophils. GINA 2026 emphasizes that the strongest predictor of a favorable therapeutic response to these agents is an elevated blood eosinophil count. *Benralizumab* is distinguished by its ability to induce near-complete eosinophil depletion through an antibody-dependent cellular cytotoxicity mechanism.

Depemokimab is presented in GINA 2026 as a novel ultra-long-acting biologic administered twice yearly, representing a significant advancement in long-term severe asthma management.

Dupilumab is an anti-IL4Rα therapy that blocks IL-4 and IL-13 signaling pathways. GINA 2026 recommends *dupilumab* in patients with T2-high asthma, elevated FeNO, eosinophilia, oral corticosteroid dependence, and comorbidities such as nasal polyps or atopic dermatitis. Its strong steroid-sparing effect and its ability to reduce the frequency of severe exacerbations are specifically emphasized. Predictors of a favorable therapeutic response include elevated FeNO and increased blood eosinophil count (BEC).

Tezepelumab is the first anti-TSLP therapy and, according to GINA 2026, holds particular importance because it acts on upstream inflammatory mechanisms. Unlike other biologics, tezepelumab demonstrates efficacy even in patients with lower T2 biomarker levels, thereby broadening therapeutic options for patients with mixed or T2-low phenotypes. GINA 2026 states that tezepelumab reduces exacerbations across a broad spectrum of patients with severe asthma regardless of eosinophil levels.

The selection of biologic therapy according to GINA 2026 is based on an individualized assessment of the patient, including phenotype, biomarkers, exacerbation frequency, comorbidities, and the need for systemic corticosteroids. In patients with allergic asthma and elevated IgE, *omalizumab* is preferred. In severe eosinophilic asthma with elevated blood eosinophils, *mepolizumab*, *benralizumab*, or other anti-

IL5/IL5R therapies are recommended. In patients with elevated FeNO, T2-high inflammation, oral corticosteroid dependence, or nasal polyps, GINA 2026 recommends *dupilumab*. In patients with mixed phenotypes, lower T2 biomarkers, or broader inflammatory activation, *tezepelumab* is recommended. GINA emphasizes that biologic selection should be an integral component of a precision medicine approach in severe asthma.

Assessment of therapeutic response according to GINA 2026 should be performed approximately 4–6 months after initiation of biologic therapy. Parameters to be evaluated include exacerbation frequency, need for systemic corticosteroids, asthma control assessed by ACT score, lung function, quality of life, and changes in biomarkers such as FeNO and eosinophil levels. GINA 2026 recommends that in the absence of an adequate therapeutic response, reassessment of the patient's phenotype, reevaluation of adherence, and, if necessary, switching to another biologic therapy should be considered. It is specifically emphasized that lack of response to one biologic does not exclude the possibility of a favorable response to another biologic with a different therapeutic target.

GINA 2026 also gives particular attention to the role of comorbidities in biologic selection. In patients with chronic rhinosinusitis with nasal polyps, dupilumab and mepolizumab are preferred. In the presence of atopic dermatitis, dupilumab has an additional advantage because of its effects on the IL-4/IL-13 pathways. In patients with a clearly allergic phenotype and allergic comorbidities, omalizumab is preferred. GINA emphasizes that proper assessment of comorbidities is a key component of individualized biologic selection and may significantly improve overall therapeutic outcomes.

Conclusions

The contemporary approach to severe bronchial asthma is based on the principles of personalized medicine and precise assessment of the patient's inflammatory phenotype. Biologic therapies have significantly changed the prognosis of patients with severe asthma by reducing exacerbations, decreasing the need for systemic corticosteroids, improving lung function, and enhancing quality of life. The central role of Type 2 inflammation, biomarkers, and comorbidities is essential in the selection of the appropriate biologic agent. An individualized therapeutic approach, regular reassessment of treatment response, and proper patient selection are considered fundamental for achieving optimal disease control. The development of new biologic therapies and the expanding possibilities for targeted treatment outline the future of severe asthma management as increasingly precise, effective, and directed toward the specific inflammatory endotype of each patient.

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Comorbidities in Heart failure with preserved ejection fraction

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Heart failure with preserved ejection fraction (HFpEF) accounts for approximately half of all heart failure cases and is characterized by marked clinical heterogeneity and complex multifactorial pathophysiology. The presence of multiple cardiometabolic and systemic comorbidities is a key element in the development and progression of the syndrome. **Aim:** To analyze the most common comorbidities in HFpEF and their relationship with the pathophysiological mechanisms of diastolic dysfunction. **Materials and methods:** A systematic review was conducted of current guidelines, consensus documents, randomized and cohort clinical trials, as well as meta-analyses focused on comorbidities in HFpEF. **Discussion:** Arterial hypertension, diabetes mellitus, coronary artery disease, atrial fibrillation, chronic obstructive pulmonary disease, obesity, and chronic kidney disease represent the major comorbidities in HFpEF. They directly contribute to pathogenesis through mechanisms such as myocardial stiffness, chronic inflammation, endothelial dysfunction, and chronic hypoxia, leading to elevated left ventricular filling pressures and progressive diastolic dysfunction. Despite therapeutic advances with sodium glucose cotransporter 2 inhibitors (SGLT2i), optimal management of comorbidities remains a fundamental factor in improving quality of life and reducing hospitalizations. **Conclusion:** HFpEF is a heterogeneous clinical syndrome in which comorbidities play a central pathophysiological and prognostic role. Due to pronounced phenotypic variability, a phenotype-based and comorbidity-oriented diagnostic and therapeutic approach is required.

Keywords: *HFpEF, Heart failure with preserved ejection fraction, Diastolic dysfunction, SGLT2 inhibitors, Comorbidities*

Introduction

Heart failure with preserved ejection fraction (HFpEF) currently accounts for approximately half of all heart failure cases and represents one of the greatest challenges in contemporary cardiovascular medicine. Unlike heart failure with reduced ejection fraction, HFpEF is characterized by marked clinical heterogeneity and a complex multisystem pathophysiology involving cardiovascular, renal, metabolic, inflammatory, and pulmonary mechanisms (Borlaug et al., 2023; Pfeffer et al., 2019).

Current evidence suggests that comorbidities play a central role in HFpEF development and progression. Hypertension, obesity, type 2 diabetes mellitus (T2DM), chronic kidney disease (CKD), atrial fibrillation (AF), chronic obstructive pulmonary disease (COPD), coronary microvascular dysfunction (CMD), and iron deficiency induce chronic systemic inflammation, endothelial dysfunction, oxidative stress, and myocardial fibrosis, ultimately leading to progressive diastolic dysfunction and elevated filling pressures (Tzoumas et al., 2025; Paolisso et al., 2024).

The marked phenotypic heterogeneity of HFpEF explains the limited efficacy of many conventional therapeutic strategies and supports the need for a phenotype-oriented and comorbidity-focused approach (Anker et al., 2023; Peters et al., 2022). The aim of the present review is to summarize the major comorbidities associated with HFpEF, their pathophysiological interactions, and their clinical implications.

Materials and methods

A systematic literature review was conducted focusing on the pathophysiology, phenotypic heterogeneity, and comorbidity burden in HFpEF.

Pathophysiology of HFpEF

HFpEF is characterized by symptoms and signs of heart failure despite preserved left ventricular ejection fraction. The syndrome

develops through complex interactions between cardiac and extracardiac abnormalities driven primarily by comorbidities. Chronic systemic inflammation induced by hypertension, obesity, diabetes mellitus, CKD, and COPD promotes endothelial dysfunction, reduced nitric oxide bioavailability, impaired cGMP-protein kinase G signaling, cardiomyocyte hypertrophy, myocardial fibrosis, and increased ventricular stiffness (Pfeffer et al., 2019; Tzoumas et al., 2025).

Coronary microvascular dysfunction, impaired myocardial energetics, left atrial remodeling, and skeletal muscle abnormalities further contribute to elevated filling pressures and exercise intolerance (Paolisso et al., 2024; Reddy et al., 2020).

Arterial hypertension

Arterial hypertension is the most common comorbidity in HFpEF and is present in more than 70–80% of patients (Pfeffer et al., 2019; Matsumoto et al., 2026). Chronic pressure overload induces structural and functional myocardial changes that progressively lead to diastolic dysfunction.

Persistent elevation of afterload promotes concentric left ventricular hypertrophy, increased myocardial stiffness, and impaired ventricular compliance (Gallo & Savoia, 2024). Activation of the renin-angiotensin-aldosterone system and sympathetic nervous system contributes to cardiomyocyte hypertrophy, collagen deposition, interstitial fibrosis, and vascular remodeling.

Hypertension also directly affects the coronary microcirculation by inducing endothelial dysfunction, reduced capillary density, and impaired coronary flow reserve, thereby contributing to chronic subendocardial ischemia and worsening myocardial relaxation (Paolisso et al., 2024).

Long-standing hypertension promotes left atrial remodeling and predisposes patients to atrial fibrillation, further aggravating filling pressures and symptom burden (Reddy et al., 2020). Inadequate blood pressure control is associated with increased hospitalization and mortality risk in HFpEF patients (Matsumoto et al., 2026).

Obesity and the cardiometabolic HFpEF phenotype

Obesity is one of the principal drivers of the cardiometabolic HFpEF phenotype and is present in the majority of HFpEF patients (Packer & Kitzman, 2018). Visceral and epicardial adipose tissue act as metabolically active inflammatory organs releasing cytokines and adipokines that induce systemic inflammation, endothelial dysfunction, oxidative stress, and coronary microvascular abnormalities (Valero-Muñoz et al., 2025).

Increased epicardial adipose tissue volume correlates with elevated filling pressures, right ventricular remodeling, and impaired exercise tolerance (Nakamori et al., 2023). Obesity-related HFpEF is additionally characterized by plasma volume expansion, sodium retention, impaired ventricular distensibility, skeletal muscle dysfunction, and increased pericardial restraint (Packer & Kitzman, 2018). Experimental studies suggest that obesity induces a distinct inflammatory and metabolic HFpEF endotype associated with adipose tissue dysfunction and impaired myocardial energetics (Daou et al., 2025; Zhou et al., 2025).

Type 2 diabetes mellitus

T2DM is among the most prevalent comorbidities in HFpEF and significantly increases the risk of developing the syndrome independently of coronary artery disease (Pfeffer et al., 2019).

Diabetes induces complex metabolic and inflammatory alterations involving insulin resistance, glucotoxicity, lipotoxicity,

oxidative stress, mitochondrial dysfunction, and accumulation of advanced glycation end-products. These mechanisms promote collagen cross-linking, extracellular matrix stiffening, endothelial dysfunction, and coronary microvascular impairment (Tzoumas et al., 2025).

Patients with diabetes frequently exhibit myocardial hypertrophy, impaired myocardial energetics, increased fibrosis, and reduced ventricular compliance (Gallo & Savoia, 2024). Diabetes is strongly associated with obesity-related HFpEF and commonly coexists with chronic kidney disease and visceral adiposity.

Chronic kidney disease

CKD is one of the most prevalent and prognostically significant comorbidities in HFpEF and represents a central component of the cardiorenal-metabolic continuum. Renal dysfunction is consistently associated with increased mortality, recurrent heart failure hospitalizations, impaired exercise capacity, and reduced quality of life in HFpEF patients (Packer et al., 2025; Li et al., 2025). Importantly, declining renal function may actively contribute to HFpEF pathogenesis through complex hemodynamic, neurohormonal, inflammatory, and metabolic mechanisms.

The relationship between HFpEF and CKD is bidirectional and self-perpetuating. Elevated venous pressure and impaired renal perfusion worsen kidney function, while CKD promotes sodium retention, plasma volume expansion, neurohormonal activation, oxidative stress, endothelial dysfunction, arterial stiffening, and chronic systemic inflammation. Together, these abnormalities contribute to ventricular-vascular uncoupling, myocardial remodeling, and progressive diastolic dysfunction.

Recent evidence suggests that visceral adiposity may represent a mechanistic link between CKD and HFpEF through adipokine imbalance, chronic inflammation, insulin resistance, and profibrotic signaling pathways affecting both the myocardium and renal parenchyma (Packer et al., 2025). In addition, CKD exacerbates coronary microvascular dysfunction and impairs myocardial energetics through endothelial injury, mitochondrial dysfunction, reduced nitric oxide bioavailability, and accumulation of uremic toxins. The emergence of SGLT2 inhibitors has substantially altered the therapeutic landscape of HFpEF by simultaneously targeting cardiovascular and renal pathways, further supporting the central role of cardiorenal interactions in HFpEF pathophysiology.

Atrial fibrillation

AF is highly prevalent in HFpEF and affects approximately 40–60% of patients, reflecting the profound structural and functional remodeling of the left atrium associated with chronically elevated filling pressures (Reddy et al., 2020). Increasing evidence suggests that AF in HFpEF should not be regarded solely as a comorbidity but rather as an integral component of disease progression and phenotypic evolution.

Chronically increased left ventricular filling pressures promote left atrial enlargement, atrial fibrosis, impaired reservoir and conduit function, and progressive atrial myopathy, thereby creating a substrate for AF development. In turn, AF further worsens ventricular filling by eliminating atrial systole, shortening diastolic filling time, increasing beat-to-beat variability, and aggravating left atrial dysfunction. This bidirectional interaction establishes a vicious cycle that accelerates hemodynamic deterioration and symptom progression.

Recent studies have demonstrated that

patients with HFpEF and AF exhibit distinct pathophysiological characteristics compared with HFpEF patients in sinus rhythm, including more advanced right ventricular dysfunction, pulmonary vascular abnormalities, impaired exercise hemodynamics, and greater systemic congestion (Reddy et al., 2020). Moreover, paroxysmal AF has been identified as an independent predictor of persistent diastolic dysfunction and impaired reversibility of abnormal filling patterns in patients with preserved ejection fraction (Kim et al., 2024), suggesting that even intermittent atrial arrhythmias may contribute to irreversible myocardial remodeling.

Beyond hemodynamic impairment, AF in HFpEF is increasingly associated with systemic inflammatory activation, endothelial dysfunction and neurohormonal dysregulation. These findings support the concept that AF and HFpEF frequently represent parallel manifestations of a common cardiometabolic and inflammatory substrate rather than isolated coexisting disorders.

Although rhythm control strategies, catheter ablation, and aggressive management of modifiable risk factors may improve symptoms and functional status in selected HFpEF patients, the optimal therapeutic approach remains incompletely defined. Future studies should focus on identifying HFpEF phenotypes most likely to benefit from early rhythm-control interventions and integrated atrial-targeted therapies.

COPD and HFpEF

Chronic obstructive pulmonary disease is a common comorbidity in HFpEF and significantly complicates diagnosis and treatment (Mooney et al., 2021; Corneanu et al., 2025). COPD contributes to chronic hypoxemia, pulmonary vascular remodeling, systemic inflammation, and increased right ventricular afterload. Meta-analytic evidence confirms that COPD is associated with higher hospitalization and mortality risk in HFpEF (Xu et al., 2024).

Pulmonary hyperinflation reduces venous return and impairs ventricular filling, thereby worsening hemodynamics in patients with limited ventricular compliance (Magrì et al., 2025). Patients with both HFpEF and COPD typically exhibit more severe dyspnea, poorer exercise tolerance, higher NYHA class, and increased cardiovascular mortality (Mooney et al., 2021). The global burden of heart failure analyses also identify COPD as one of the important underlying contributors to heart failure burden worldwide (Ran et al., 2025).

Coronary microvascular dysfunction

CMD is increasingly recognized as a central pathophysiological mechanism in HFpEF and may be present in up to 75% of patients without obstructive epicardial coronary disease (Tzoumas et al., 2025).

Comorbidities such as hypertension, diabetes, obesity, and CKD induce endothelial dysfunction, oxidative stress, reduced nitric oxide bioavailability, capillary rarefaction, and impaired coronary flow reserve. These abnormalities result in chronic subendocardial ischemia, impaired myocardial energetics, increased myocardial stiffness, and progressive fibrosis (Paolisso et al., 2024).

Iron deficiency and anemia

Iron deficiency and anemia are common in HFpEF and significantly contribute to impaired exercise capacity, fatigue, and poor quality of life (Alnuwaysir et al., 2022). Iron deficiency may occur independently of anemia and is associated with chronic inflammation, hepcidin dysregulation, impaired gastrointestinal absorption, and renal dysfunction. Anemia in heart failure is multifactorial and may reflect renal dysfunction, iron

deficiency, impaired erythropoietin production, nutritional abnormalities, and systemic inflammatory activation (Grote Beverborg et al., 2018).

Because iron plays a critical role in mitochondrial function and skeletal muscle energetics, deficiency contributes to reduced oxygen utilization, skeletal muscle dysfunction, and impaired myocardial metabolism (Kido et al., 2024). These mechanisms explain why iron deficiency may worsen functional limitation even in the absence of overt anemia (Alnuwaysir et al., 2022; Kido et al., 2024).

Future therapeutic strategies

Future therapeutic strategies in HFpEF include both pharmacological and device-based approaches targeting the specific pathophysiological mechanisms and clinical phenotypes of the syndrome. In recent years, several interventional modalities have been investigated, including interatrial shunt devices, vagal nerve stimulation, baroreflex activation therapy, cardiac contractility modulation, renal denervation, catheter ablation, and greater splanchnic nerve ablation, with the aim of reducing left ventricular filling pressures, improving hemodynamics, and alleviating symptoms in patients with HFpEF (Araz et al., 2025). However, despite promising preliminary findings and demonstrated procedural feasibility in multiple early-phase trials, current evidence remains insufficient to support widespread clinical implementation of these therapies. The heterogeneous nature of HFpEF likely contributes to the variable therapeutic response observed across studies and highlights the importance of more precise phenotype-based patient selection in future clinical trials. In parallel, ongoing experimental and translational research continues to emphasize the role of pulmonary vascular remodeling, systemic inflammation, metabolic dysfunction, and coronary microvascular abnormalities in HFpEF progression. These findings underscore the need for improved preclinical models capable of accurately reproducing the cardiometabolic and inflammatory substrate of the syndrome in humans, which may facilitate the development of more targeted and individualized therapeutic approaches in the future (Dai & Thorp, 2023).

Conclusion

HFpEF represents a complex and heterogeneous multisystem syndrome in which comorbidities are not merely associated conditions but fundamental drivers of disease initiation, progression, and clinical expression. Hypertension, obesity, diabetes mellitus, chronic kidney disease, atrial fibrillation, COPD, coronary microvascular dysfunction, and iron deficiency interact through overlapping mechanisms involving systemic inflammation, endothelial dysfunction, oxidative stress, myocardial fibrosis, impaired ventricular-vascular coupling, and metabolic dysregulation. These interconnected pathways ultimately converge toward elevated filling pressures, impaired myocardial reserve, and severe exercise intolerance.

Contemporary evidence strongly supports the paradigm shift from viewing HFpEF as a single disease entity toward recognizing it as a spectrum of overlapping clinical and biological phenotypes. This concept may explain the heterogeneous therapeutic responses observed in previous clinical trials and highlights the limitations of a “one-size-fits-all” treatment strategy. Future progress in HFpEF management will likely depend on improved phenotypic characterization, integration of multimodal biomarkers and imaging, and development of mechanism-based individualized therapeutic approaches.

Although SGLT2 inhibitors represent a major therapeutic breakthrough in HFpEF, substantial residual morbidity and mortality persist, particularly among patients with extensive multimorbidity burden. Consequently, optimal management of comorbidities remains central to improving outcomes in HFpEF. Further translational and clinical research is required to clarify phenotype-specific mechanisms, refine patient stratification, and identify novel therapeutic targets capable of modifying the underlying cardiometabolic and inflammatory substrate of the syndrome.

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Microbiological causative agents of infective endocarditis: a 17-year retrospective study

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Introduction: The profile of infective endocarditis (IE) continues to evolve over recent decades. Routine implantation of valve prostheses, prosthetic materials for valve and vascular reconstruction, intracardiac devices, and catheters changes the etiological spectrum of the disease and influences the distribution of causative microorganisms. **Aim:** We aim to identify and analyze the microbiological etiological agents of infective endocarditis. **Materials and methods:** This single-center retrospective study includes 270 patients treated at the University Hospital "St. George" – Plovdiv between January 2005 and December 2021. **Results:** Staphylococci spp. represent the most common pathogens (33.3%), with Staphylococcus aureus accounting for 16.5% and coagulase-negative staphylococci (CoNS) for 16.8%. Enterococci spp. account for 9.4%: Enterococcus faecalis – 8.6%, Enterococcus durans – 0.4%, and other Enterococcus species – 0.4%. Streptococci spp. represent 7.8%, including Streptococcus viridans – 3.4%, Streptococcus beta-hemolyticus – 0.7%, Streptococcus alpha-hemolyticus – 2.2%, and other Streptococci – 1.5%. Non-HACEK gram-negative microorganisms constitute 7.1%, with Escherichia coli (3.4%) being the most frequent. Other pathogens remain below 2%. Negative blood cultures show a high proportion of 40.4%. **Discussion:** The microbiological profile in our cohort reflects global trends. Staphylococci remain the leading cause of IE. The proportion of Streptococci continues to decline, while Enterococci and gram-negative microorganisms show an increasing trend. These shifts correspond to the rising incidence of healthcare-associated IE, IE related to intravenous drug use, and fewer cases linked to dental sources. **Conclusion:** Understanding the microbiological etiological agents of infective endocarditis remains essential for timely diagnosis, prevention, and effective management of this life-threatening condition.
Keywords: *infective endocarditis, microbiological etiological agents*

Introduction

The profile of infective endocarditis (IE) has changed significantly over the past few decades. The routine implantation of prosthetic valves, prosthetic materials for valve and vessel reconstruction, intracardiac devices, and catheters has changed the etiological spectrum of the disease. The emergence of healthcare-associated IE has been accompanied by an increase in the prevalence of Staphylococcus aureus and coagulase-negative staphylococci, while the proportion of IE cases caused by Streptococcus viridans has decreased significantly. Enterococci are now the third most common cause of IE and are becoming increasingly prevalent in healthcare-associated IE. As recent recommendations for the treatment of IE are largely based on non-randomized studies and expert opinion, empirical antibiotic therapy is usually administered according to local microbiological characteristics. For this reason, it is important to periodically update information on the characteristics of regional IE pathogens and their antibiotic susceptibility profiles.

Objective

The aim of our study is to identify the microbiological agents that cause IE in our region.

Material and methods

The study is single-center, retrospective and includes 270 patients with definite or probable IE according to the Duck diagnostic criteria, treated at the University Hospital "St. Georgi" – EAD, Plovdiv for the period January 2005 – December 2021. We divided the patients into two groups – alive (203) and deceased (67) in order to look for a

significant difference in the causative agents. Microbiological studies were conducted in the Microbiology Laboratory of the University Hospital "St. Georgi" – EAD, according to established standards. Ready-made bottles with liquid nutrient media BacT/ALERT (bioMerieux, France) were used, allowing the cultivation of aerobes, anaerobes and fungi. The statistical analysis was performed with the statistical package of the social sciences software IBM SPSS Statistics v. 26. For all tests, a significance level of $\alpha < 0.05$ was adopted.

Results

Of the diagnosed microorganisms, the largest share is the group Staphylococci spp – 33.3%, with Staphylococcus aureus being 16.5% and Staphylococcus CoNS being 16.8% of the total number of patients. The following are the group Enterococci spp. – 9.4%, respectively for Enterococcus species – 0.4%, Enterococcus faecalis – 8.6% and Enterococcus durans – 0.4%. For Streptococci spp – 7.8%, distributed as follows: Streptococcus viridans – 3.4%, Streptococcus beta-hemolyticus – 0.7%, Streptococcus alfa hemolyticus – 2.2% and other Streptococci – 1.5%. Group of gram-negative microorganisms, non-HASEK – 7.1%, of which Pseudomonas aeruginosa – 0.7%, Escherichia coli 3.4%, Enterobacter cloacae – 0.4%, Klebsiella pneumoniae – 1.1%, Serratia marcescens – 1.5%. Other microorganisms make up less than 2%. The share of negative blood cultures is high – 40.4% (Table 1).

Table 1. Microbiological agents

Microbiological agents, n (%)	Total n = 270 G0	Deceased n = 67 G3	Alive n = 203 G4	p-value*
Отрицателни хемокултури	108 (40.4)	23 (34.3)	85 (41.9)	0.271
Staphylococci	88 (33.3)	21 (31.3)	68 (33.5)	0.740
Staphyloc. aureus	44 (16.5)	10 (14.9)	34 (16.7)	0.730
Staphylococcus CoNS	45 (16.8)	11 (16.4)	34 (16.8)	0.939
Streptococci	21 (7.8)	3 (4.5)	18 (7.0)	0.468
Streptococcus viridans	8 (3.0)	2 (3.0)	6 (3.0)	1.000
Streptococcus mitis	1 (0.4)	0 (0.0)	1 (0.5)	0.562
Streptococcus beta-hemolyt	2 (0.7)	0 (0.0)	2 (1.0)	0.411
Streptococcus alfa hemolyt	6 (2.2)	1 (1.5)	5 (2.5)	0.632
Streptococci - други	4 (1.5)	0 (0.0)	4 (2.0)	0.244
Enterococci	25 (9.4)	8 (11.9)	17 (8.4)	0.392
Enterococcus species	1 (0.4)	0 (0.0)	1 (0.5)	0.562
Enterococus faecalis	23 (8.6)	8 (11.9)	15 (7.4)	0.253
Enterococcus durans	1 (0.4)	0 (0.0)	1 (0.5)	0.562
Gram negativni (non-HASEK)	19 (7.1)	11 (15)	9 (4.5)	**0.004**
Pseudomonas aeruginosa	2 (0.7)	1 (1.5)	1 (0.5)	0.410
Escherichia coli	9 (3.4)	5 (7.5)	4 (2.0)	**0.030**
Enterobacter cloacae	1 (0.4)	0 (0.0)	1 (0.5)	0.562
Klebsiella pneumoniae	3 (1.1)	1 (1.5)	2 (1.0)	0.736
Serratia marcescens	4 (1.5)	3 (4.5)	1 (0.5)	**0.019**
Others	5 (1.9)	2 (3.0)	3 (1.5)	0.432
Candida spp	3 (1.1)	1 (1.5)	2 (1.0)	0.736
Erysipelothix rhusiopathiae	1 (0.4)	0 (0.0)	1 (0.5)	0.562
Brevibacterium casei	1 (0.4)	1 (1.5)	0 (0.0)	0.080

z – test*

Our data shows that Gram negative (non-HASEK) IE makes up 7.1% of cases, with a significantly higher mortality rate for the whole group and for the microorganisms Escherichia coli and Serratia marcescens (see Fig. 1).

Discussion

The microbiological results in our sample are comparable to those reported in almost all current studies. The EURO-ENDO results showed the following percentages for different types of bacteria: 44.1% for Staphylococci, 15.8% for Enterococci, 12.3% for Streptococci, and 3.5% for GNB non-HASEK (5). The data for ICI-PCS show that 42% of cases are due to Staphylococci, 10% to Enterococci and 31% to Streptococci (6). In France, the figures are 43.9% for staphylococci, 44.6% for enterococci and streptococci together and 9.1% for GNB non-HASEK (7). The data for Latin America are as follows: 53.8% for staphylococci; 9.3% for enterococci; 26.7% for streptococci; and 2.8% for GNB non-HASEK (8). The data for Africa are as follows: 41.3% for staphylococci; 9.1% for enterococci; 34% for streptococci; and 10.6% for GNB non-HASEK (9). The data for Portugal are as follows: 16.4% for staphylococci; 7.1% for enterococci; 13.6% for streptococci; and 11.9% for GNB non-HASEK (10). The data for Canada are as follows: 37.8% for staphylococci; 12.8% for enterococci; and 25.5% for streptococci (11). The proportions are as follows: Japan: 38.9% for staphylococci, 7.8% for enterococci, 22.2% for streptococci (12). Iran: 27.7% for staphylococci, 8.3% for enterococci, 8.3% for streptococci, 9.5% for GNB non-HASEK (13).

South Korea: 6% for staphylococci, 7% for enterococci, 9.1% for streptococci (55). South Korea is an exception, with streptococci being the most prevalent causative agent at 35.1%, followed by staphylococci at 23.4% and enterococci at 8.6%. Contemporary registries show that the leading causative agent of IE is staphylococci, with a decrease in the proportion of streptococci and an increase in the proportion of enterococci and gram-negative microorganisms. This is directly related to an increase in IE cases associated with healthcare and IE resulting from intravenous drug abuse, as well as a decrease in cases involving a dental portal of infection.

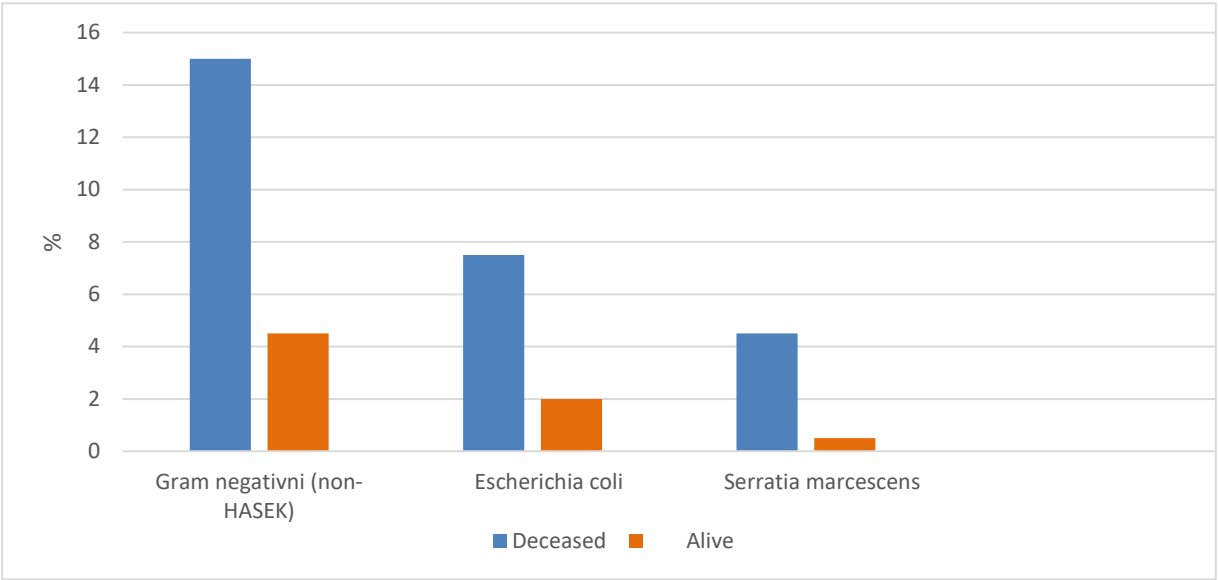

Figure 1. Gram-negative non-HACEK bacteria in deceased and alive

The proportion of negative blood cultures varies significantly (10–52%) and is primarily associated with previous prolonged antibiotic treatment. Our data show negative blood cultures in 40.4% of patients and previous antibiotic treatment in 53.6%.

Gram-negative non-HACEK infective endocarditis (NHGNIE) is rare, accounting for approximately 1.5–10.7% of IE cases worldwide. The most common pathogens are Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae and Escherichia coli. These bacteria are usually multidrug-resistant, which makes treatment difficult and results in a high mortality rate (15%). NHGNIE is more prevalent among intravenous drug users and immunosuppressed patients, particularly in cases of prosthetic and device-related IE. Our data are consistent with those reported in the literature.

Serratia marcescens is most commonly associated with intravenous drug use, but it can also affect immunocompromised patients. The bacterium is known for its ability to form biofilms and produce beta-lactamase, which leads to resistance to standard antibiotics. This resistance complicates treatment and results in a high mortality rate. In our study, the mortality rate was 75%, consistent with previous research. Early recognition and effective treatment are crucial for improving outcomes in patients with endocarditis caused by Serratia marcescens (16, 17).

Conclusion

Knowledge of the microbiological causative agents of IE is of utmost importance for both the prevention and treatment of this life-threatening disease.

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Cardiac involvement in dermatomyositis – a case report

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Dermatomyositis (DM) is a type of idiopathic inflammatory myopathy (IIM) with accompanying skin rashes. There are observations about increased incidence of cardiovascular disorders including arrhythmias, heart failure, coronary artery disease in IIMs. Here, we present a 57-year-old woman with confirmed diagnosis DM (based on the presence of proximal muscle weakness of upper and lower extremities, elevated muscle enzymes, electromyographic and histological confirmation of myositis, positive ANA and anti-Mi2 antibody). The patient presented in our department with clinical worsening of proximal muscle weakness and the patient needed foreign help for her everyday activities. In addition, there was symmetric arthritis, heliotrope rash and Gottron sign. She also reported about dysphagia, dyspnea, palpitations and presyncope. She received a beta-blocker (5 mg bisoprolol) for mild accompanying arterial hypertension. The value of creatine phosphokinase was 3336, lactate dehydrogenase – 2329. Computed tomography of the lungs demonstrated presence of interstitial lung disease. Methylprednisolon at the dose of 1 mg/kg was started. Holter monitoring revealed episodes of atrial fibrillation and frequent ventricular extrasystoles up to trigeminy. The beta-blocker was combined with flecainide 100 mg daily and anticoagulant with excellent control on clinical symptoms without residual complaints. Follow-up of the patient is for one year until now with achieved stable remission of the disease. Methylprednisolon was gradually tapered to 10 mg daily accompanied by combined antiarrhythmic and anticoagulant treatment. Optimal management of cardiac manifestations in DM patients requires multidisciplinary care. Further studies are necessary to delineate their associations with disease activity and establish precise diagnostic and therapeutic protocol.

Introduction

IIMs are rare autoimmune diseases characterized by symmetrical proximal muscle weakness. They are a heterogeneous group, including dermatomyositis (DM), polymyositis, inclusion body myositis, paraneoplastic myositis, etc. (1). Dermatomyositis (DM) is a rare type of IIM, characterised mainly by skeletal muscle inflammation and specific skin lesions (1), (2). The clinical manifestations may vary from classical DM, juvenile DM, paraneoplastic, overlap myositis, hypo- and amyopathic DM (1), (3). DM may also involve other organs and systems such as the heart, lungs, and gastrointestinal tract. The pathognomonic skin lesions of classic DM include Gottron's papules and Gottron's sign – papules or erythematous macules over metacarpophalangeal, proximal, and distal interphalangeal joints. Periungual regions could be involved. Characteristic skin findings include heliotrope rash (violaceous erythema, at times with oedema, affecting the upper eyelids), shawl sign, V-sign, etc. (1). Cardiac involvement in DM presents with non-specific signs and may be subclinical. It indicates poor prognosis and is a major cause of mortality in these patients (4). This case highlights the extensive clinical spectrum of DM and reinforces the importance of considering cardiac involvement during patient evaluation, as early detection and management improve prognosis.

Case presentation

A 57-year-old woman with a confirmed diagnosis of DM presented in the department with clinical worsening of proximal muscle weakness, dysphagia, dyspnea, palpitations, and pre-syncope. Physical examination showed symmetric arthritis, heliotrope rash, and Gottron's sign.

The diagnosis was confirmed based on proximal muscle weakness, positive antinuclear antibodies (ANA) and anti-Mi2 antibodies, electromyographic and histological confirmation of myositis, and elevated muscle enzymes (5). The value of creatine phosphokinase was 3336, and lactate dehydrogenase was 2329. Holter monitoring demonstrated episodes of atrial fibrillation and frequent ventricular extrasystoles up to trigeminy. Furthermore, computed tomography of the lungs demonstrated interstitial lung disease.

The patient in this case presented with symptoms of pre-syncope and palpitations, prompting for further investigations, which confirmed ventricular extrasystoles up to trigeminy and atrial fibrillation. Treatment with methylpredisnisolone at a dose of 1 mg/kg was initiated leading to a clinical improvement and eventually the dose was tapered down to 10 mg daily, whilst maintaining remission. Managing therapy with corticosteroids whilst minimising cardiac side effects can be challenging: it is important to closely monitor these patients. To manage the cardiac complications, bisoprolol as well as flecainide at a dose of 100 mg was administered. In addition, anti-coagulation therapy was initiated to prevent any potential thromboembolic complications that could arise from the atrial fibrillation.

Discussion

Current knowledge shows that in patients with IIMs myocardial involvement is among the leading causes of increased mortality (4), (6). Histologically, DM is a microangiopathy with capillary damage and secondary ischemic alterations in muscle fibers, while in polymyositis cellular infiltrates (CD8+ lymphocytes) with predominant endomysial localization are detected. It is assumed that immune-mediated inflammation may also affect myocardium and even the conducting system. This notion is supported by observation of mononuclear inflammatory infiltrates localized in the endomysium and perivascular areas, degeneration of cardiac myocytes, myocarditis, fibrosis. Vasculitis of the coronary arteries has been reported, as well as severe atherosclerosis. Other possible causes of cardiac pathology should be considered, including advanced coronary artery disease. 70% of cases with cardiac involvement are subclinical, and only 10% of cases present overtly (6).

In cases of cardiac pathology in IIM, electrocardiographic and Holter abnormalities include atrial or ventricular premature beats, atrial tachycardia, ventricular tachycardia, atrial fibrillation, atrioventricular conduction block, bundle branch blocks, abnormal Q-waves, ST-T wave changes. Echocardiography is standardly used technique to assess presence of systolic or diastolic cardiac dysfunction. Cardiac magnetic resonance is considered the best non-invasive tool as it enables the detection of both active inflammation and chronic fibrosis. Additional investigations to assess cardiac damage include scintigraphy with Technetium 99m-pyrophosphate (99mTc-PYP) (6). 18 F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) could be also used for early detection of cardiac involvement (7).

Conclusion

Dermatomyositis may present with extramuscular involvement, including rare cardiac complications. An early and thorough evaluation of the patient can significantly improve the patient's outcome and can decrease the morbidity and mortality. The lack of standardised protocols for screening and managing cardiac complications makes treatment decisions difficult. Managing

immunosuppressive therapy for muscle inflammation while minimising cardiac side effects can be challenging. High clinical suspicion and detailed assessment are essential. Early recognition and appropriate management can improve patient outcomes. This case emphasises the value of multidisciplinary collaboration in complex autoimmune disorders.

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Recurrent drug-induced angioedema – a clinical case

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Introduction: Drug-induced angioedema is a serious clinical condition due to its variable presentation and the risk of upper airway obstruction. Differential diagnosis between histamine-mediated and bradykinin-mediated mechanisms is critical for the correct therapeutic approach. **Objective:** To analyze the clinical course, diagnostic process and therapeutic procedures in a patient with recurrent angioedema in the context of combined drug therapy and concomitant diseases. **Materials and methods:** A clinical case of a patient with arterial hypertension and prolonged cardiological therapy, hospitalized due to swelling of the tongue is presented. Laboratory, immunological and functional studies were performed, including determination of complement fractions and allergy testing. **Results and discussion:** No complement deficiency was detected. There is polysensitization to inhalant allergens unrelated to the acute episodes. The clinical picture, the absence of urticaria, and the recurrent nature of the edema point to a bradykinin mechanism associated with the intake of angiotensin-converting enzyme inhibitor and acetylsalicylic acid. Discontinuation of the provoking medications leads to stabilization of the condition. The case highlights the importance of the medication history in patients with polymorbidity. **Conclusion:** Recurrent angioedema requires a systematic diagnostic approach and timely reassessment of the therapeutic regimen. Recognition of the bradykinin mechanism is essential for the prevention of severe and potentially fatal complications.
Keywords: *angioedema, allergology, clinical case*

Introduction

Angioedema is defined as a clinical syndrome characterized by self-limiting edema of subcutaneous and/or submucosal tissue, of sudden onset, caused by increased vascular permeability. The condition is seen primarily in African-American women and older adults. [1] After 2010, following a thorough study of the phenotypes and endotypes of the disease, numerous classifications and guidelines of national and international communities and organizations in immunopathology and allergology were published. Systematized, the main types of angioedema can be presented as: hereditary angioedema – type I, type II and with normal complement, angioedema induced by angiotensin-converting enzyme inhibitors, acquired angioedema, histaminergic and non-histaminergic angioedema [2]. In some classifications and guidelines, angioedema is considered separately, in others with and without rashes, and as a specific form, hereditary angioedema is also subdivided into several types. At the pathophysiological level, the mechanism of action is related to the release of vasoactive substances, which leads to the classification of angioedema as histamine-mediated and bradykinin-mediated [2, 3]. The presence of urticaria is usually observed in histaminergic angioedema and is crucial for the correct diagnosis [4]. The etiology of drug-induced angioedema is mainly due to ACE inhibitors, with a small number of studies showing its relationship with angiotensin II receptor antagonists, psychotropic substances, fibrinolytic agents and estrogens [5]. Clinical manifestations related to the pharynx and larynx are rare, but can progress to life-threatening asphyxia [4–6]. Modern treatment of angioedema is based on the identification of its phenotype and endotype, and is personalized depending on the triggering factor, the leading pathophysiological mechanism, the localization of the edema and its clinical manifestations.

The therapeutic regimen most often includes the use of antihistamines, corticosteroids, epinephrine, and in life-threatening conditions with laryngeal localization of edema – endotracheal intubation [1]. Among the leading causes of this condition are medications, with ACE inhibitors playing a key role in recent years. Despite the fact that only 0.1 to 0.7% of the population has angioedema as an adverse drug reaction, the use of ACE inhibitors by millions of hypertensive patients worldwide has led to an increasing incidence of the disease [1, 7]. Nearly 40 million patients are treated with ACE inhibitors annually, and this leads to hundreds of deaths from laryngeal edema [5]. The potential for fatal asphyxia makes early identification of the condition critical for its timely diagnosis and adequate treatment.

Case presentation

A 73-year-old woman with complaints of swelling of the tongue and sublingual area, as well as dysphagia and dysphonia, visited the emergency department of municipal hospital on 10.03.2024. The complaints appeared about 90 minutes after consuming whole-grain pastry. The patient had a similar episode in December 2023, when symptoms appeared within 15 minutes after consuming sweets. She was hospitalized with respiratory distress and oropharyngeal edema, requiring emergency medication with intravenous methylprednisolone (40 mg twice daily) and chloropyramine (20 mg). Due to the severity of the sublingual edema, oxygen therapy via face mask was administered at a flow rate of 6–7 L/min. The refractory nature of the edema necessitated consultations with cardiology and otolaryngology specialists. For the treatment of concomitant arterial hypertension, the patient took a combined antihypertensive drug containing an ACE inhibitor and aspirin. After discharge, she was referred to the Department of Occupational Diseases with clinical allergology activity for etiological clarification and treatment. The investigated C3 and C4 fractions of complement were within reference limits. Skin allergy tests demonstrated sensitization to grass, tree pollens and micromites in house dust. A repeat consultation with a cardiologist was carried out and the multidisciplinary team of the department, having precisely examined the patient to clarify the etiology of the episodes of angioedema, proved that the edema was drug-induced, of a combined – bradykinin and metabolic type.

Discussion

The clinical case presented by us is original considering the possibility of manifestation of angioedema by combined drug-induced endotype – bradykinin and metabolic. The main mechanism involves ACE-inhibitor-induced reduction of bradykinin degradation. Its inhibition leads to accumulation of bradykinin, activation of B2-receptors, release of substance P and subsequent increase in vascular permeability, causing fluid extravasation [7]. In the patient, an ACE inhibitor was identified as a component of the antihypertensive medication she was taking, in combination with two other antihypertensive agents belonging to other pharmacological groups. Aspirin acts as a cofactor by inhibiting cyclooxygenase, reducing the protective effect of prostaglandin E2 and shifting metabolism to the leukotriene pathway. This shift has a synergistic effect on inflammation and vascular permeability. The absence of urticaria and pruritus serves as a key clinical feature distinguishing bradykinin-dependent from histamine-mediated responses. Corticosteroid resistance further supports this claim. Unlike histamine, bradykinin-mediated edema has a slower onset within a few hours and may persist for days

until it either undergoes complete spontaneous reversal or is reversed by treatment with bradykinin antagonists or plasma kallikrein-kinin inhibitors, bypassing the mast cell-dependent pathway entirely [8]. In this and similar cases, discontinuation of the edema-inducing medication is a mandatory element of the comprehensive therapeutic approach. Given the risk of laryngeal edema, ensuring a clear airway through intubation is among the measures of paramount importance for saving patients' lives [9].

Conclusion

The data from the anamnesis, subjective complaints, features of the clinical picture and paraclinical examinations are the main diagnostic criteria for the diagnosis of drug-induced angioedema. Preventive measures are of utmost importance. Prevention begins with active monitoring by general practitioners, and referral to specialist allergists is mandatory to identify the phenotype and endotype of angioedema. And they are of key importance in determining adequate therapy for this urgent and often life-threatening condition in each patient. The appropriate treatment for drug-induced angioedema remains a challenge, which further emphasizes the need for the multidisciplinary approach demonstrated in this case.

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Diagnostic importance of contrast-enhanced computed tomography in acute necrotizing pancreatitis: a case review

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Acute necrotizing pancreatitis is one of the most severe presentations of pancreatic inflammation. Pancreatic or peripancreatic tissue necrosis develops in 10–15% of cases and is associated with a prolonged clinical course, frequent local complications, and increased mortality. In clinical practice, contrast-enhanced CT (CECT) remains the most reliable modality for confirming necrosis, assessing its extent, and guiding patient management. This report describes a 46-year-old man with a history of cholecystectomy who presented with sudden epigastric pain, nausea after eating or drinking, and dark urine. Laboratory tests showed the typical pattern of elevated pancreatic enzymes and inflammatory markers. The initial CECT demonstrated a markedly enlarged pancreas with patchy, heterogeneous enhancement and broad areas of non-enhancing parenchyma, consistent with necrosis. Small islands of enhancing tissue were preserved in the tail and uncinate process. Fluid collections were also present around the pancreas, liver, and spleen, indicating combined pancreatic and peripancreatic necrosis. Using the Balthazar CT Severity Index, the findings corresponded to a score of 10, reflecting severe acute pancreatitis. Follow-up imaging was performed using non-contrast CT to assess the evolution of pancreatic enlargement and peripancreatic fluid collections. The examination demonstrated persistent but reduced inflammatory changes, correlating with clinical and laboratory improvement. This case highlights the essential role of CECT in diagnosing and managing acute necrotizing pancreatitis. CT imaging provides clarity on the extent of necrosis, helps assess severity, and offers valuable guidance during follow-up, making it a key element of care in patients with this challenging condition.

Keywords: *acute pancreatitis, necrotizing pancreatitis, pancreatic inflammation, contrast-enhanced CT*

Introduction

Acute pancreatitis is an acute inflammatory condition of the pancreas diagnosed when at least two of the following are present: characteristic abdominal pain, serum amylase and/or lipase levels exceeding three times the upper limit of normal, and imaging findings consistent with pancreatitis (1). Gallstones remain the most common cause, followed by alcohol use, while other etiologies include hypertriglyceridemia, medications, post-ERCP complications, hypercalcemia, hereditary disorders, and infections. Approximately 10–20% of cases are classified as idiopathic (1).

Acute pancreatitis is broadly classified into edematous and necrotizing forms. Necrotizing pancreatitis represents the more severe subtype, characterized by pancreatic parenchymal necrosis, peripancreatic necrosis, or both (1, 5). The Revised Atlanta Classification (2012) provides a standardized framework for severity assessment, categorizing disease as mild, moderately severe, or severe based on organ failure and complications. It also distinguishes early and late disease phases and defines the major pancreatic fluid collections encountered during progression (1). Contrast-enhanced computed tomography (CECT) plays a central role in evaluating severe or diagnostically uncertain cases (2). It is also essential for assessing pancreatic necrosis and disease severity through the CT Severity Index (CTSI), which incorporates both inflammatory changes and the extent of necrosis (2, 3). This report presents a case of severe acute necrotizing pancreatitis highlighting the diagnostic and follow-up value of CT imaging.

Case presentation

A 46-year-old man presented to the gastroenterology department with severe upper abdominal pain accompanied by nausea, vomiting, and dark urine. His medical history was notable for previous calculous cholecystitis and choledocholithiasis, for which he had undergone cholecystectomy. On admission, laboratory evaluation revealed markedly elevated pancreatic enzymes, with serum alpha-amylase of 4950 U/L and lipase of 16,880 U/L, along with elevated inflammatory markers, consistent with acute pancreatitis. A contrast-enhanced computed tomography (CECT) examination was performed. Imaging demonstrated marked enlargement of the pancreas with poorly defined margins and heterogeneous enhancement. Extensive non-enhancing areas involving the pancreatic body and head were identified. Associated peripancreatic inflammatory changes were present, together with free fluid collections extending into the peripancreatic, perihepatic, and perisplenic spaces (Figure 1A-B).

These findings were consistent with acute necrotizing pancreatitis involving both pancreatic and peripancreatic tissues. Based on the extent of necrosis and associated fluid collections, the CT Severity Index (CTSI) score was calculated as 10, indicating severe disease.

The patient was managed conservatively with supportive treatment and his clinical condition gradually improved. A follow-up non-contrast CT examination performed weeks later demonstrated still pancreatic enlargement, but decreased peripancreatic inflammatory changes, and partial resorption of the fluid collections, in keeping with favorable clinical evolution (Figure 2).

A.

B.

Figure 1. Contrast-enhanced CT (axial) demonstrating severe acute necrotizing pancreatitis. (A) Enlarged pancreas with heterogeneous enhancement and areas consistent with necrosis. (B) Associated free fluid into the perihepatic and perisplenic space.

Figure 2. Follow-up non-contrast axial CT demonstrating still pancreatic enlargement, decreased inflammatory changes, and partial resolution of the peripancreatic fluid collections.

Discussion

Acute pancreatitis remains a healthcare burden worldwide, with increasing incidence reported in Europe (4). Although most cases are mild and self-limiting, necrotizing pancreatitis represents a severe form associated with significantly higher morbidity and mortality (5). The severity of necrotizing pancreatitis is primarily related to the systemic inflammatory response triggered by tissue necrosis, which may lead to organ failure in the early phase of disease. Later complications include infected necrosis, persistent collections, and walled-off necrosis, often requiring interventional management such as drainage or necrosectomy (1, 5). CECT remains the imaging modality of choice for identifying pancreatic necrosis (2). Non-enhancing pancreatic tissue is the hallmark imaging feature, while peripancreatic fat necrosis appears as hypodense inflammatory changes. Gas within collections may suggest superinfection (2). Optimal assessment of necrosis is generally achieved after 72 hours from symptom onset, when the full extent of ischemic damage becomes more apparent (2). CT was critical for diagnosis and severity assessment.

Conclusion

This case highlights the essential role of contrast-enhanced CT in the diagnosis, severity assessment, and follow-up of acute necrotizing pancreatitis. Early recognition of extensive necrosis enables appropriate risk stratification and management.

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Neck lymph node metastases from extra-thyroid pathology – a case report

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Introduction: The ultrasound examination of the neck area is commonly used for assessment of the thyroid and parathyroid glands. The morphological evaluation of the neck lymph nodes is an important aspect of the diagnostic process. Neck lymph node metastases usually occur as a result of thyroid cancer or head and neck malignancy. Rarely distant metastases from other primary carcinoma can be seen, which can pose a diagnostic challenge. **Case presentation:** A 53-year old female with a history of longstanding diabetes was referred for hospitalization due to persistently impaired glucose control. Besides reporting weight gain, polydipsia and polyuria, overall she was in very good general condition. During a routine ultrasound examination of the neck area, multiple pathologically enlarged neck lymph nodes were visualized. A single thyroid nodule with lack of risk-features was noted. A chest x-ray revealed shadowing in the right lung, suspicious for malignancy. Bronchoscopy was performed and the histological result described low-differentiated adenocarcinoma of the lung. EGFR gene mutation (exon 19 deletion was confirmed) and the patient was started on Ozimertinib. Rapid shrinkage of the neck lymph nodes was noted shortly after the initiation of treatment. **Conclusion:** Distant metastases to the neck lymph nodes from lung cancer are uncommon. Nevertheless, it is crucial to bear in mind the possibility of such peculiarities in order to broaden the differential-diagnostic plan and reach the correct medical conclusion in a timely manner, which in cases of highly-aggressive tumors can be life-saving.

Keywords: *ultrasound, lymph node metastases, lung cancer*

Introduction

Ultrasound examination is a widely used imaging modality for the morphological assessment of the thyroid and parathyroid glands. During routine evaluation, cervical lymph nodes are also assessed. Cervical lymph node metastases are most commonly associated with thyroid carcinoma or head and neck malignancies. However, metastases from distant primary tumors may occasionally occur and pose a significant diagnostic challenge.

Case presentation and discussion

A 53-year-old female with a history of longstanding type 2 diabetes was referred for hospitalization due to persistently poor glucose control despite treatment with metformin and basal insulin. She reported weight gain, polydipsia, and polyuria, but was otherwise in good general condition. On physical examination, a firm nodule was palpated above the left clavicle. The sonographic evaluation of the neck area revealed multiple pathologically enlarged neck lymph nodes bilaterally. The lymph nodes were clustered, enlarged, round, with increased echogenicity and lack of a hilum – all features suggestive of malignancy (Fig. 1 and Fig. 2). Additionally, a single low-risk thyroid nodule was identified in the right lobe. It was isoechoic with a wide vascularized halo, without any suspicious features. (Fig. 3)

Given these findings, several differential diagnoses were considered: metastatic thyroid carcinoma, lymphoma, head and neck malignancy, metastases from an unknown primary site.

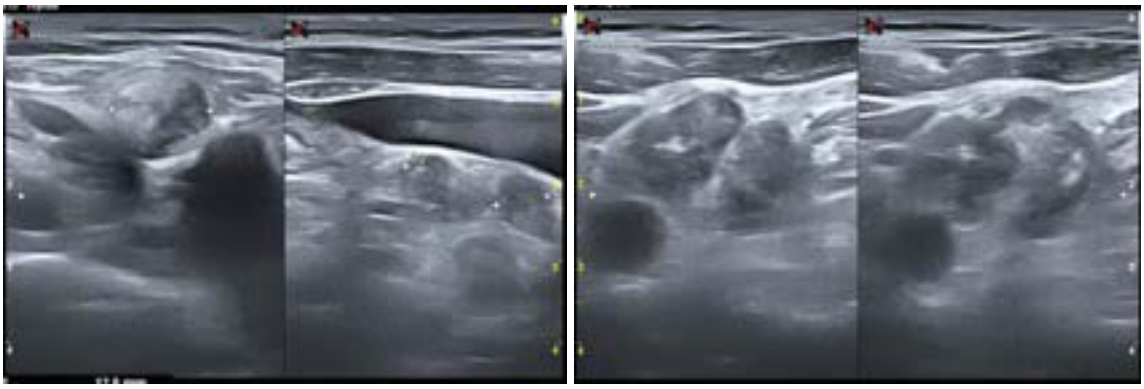

Figure 1 and **Figure 2.** Ultrasound presentation of pathological lymph nodes in the left neck compartment

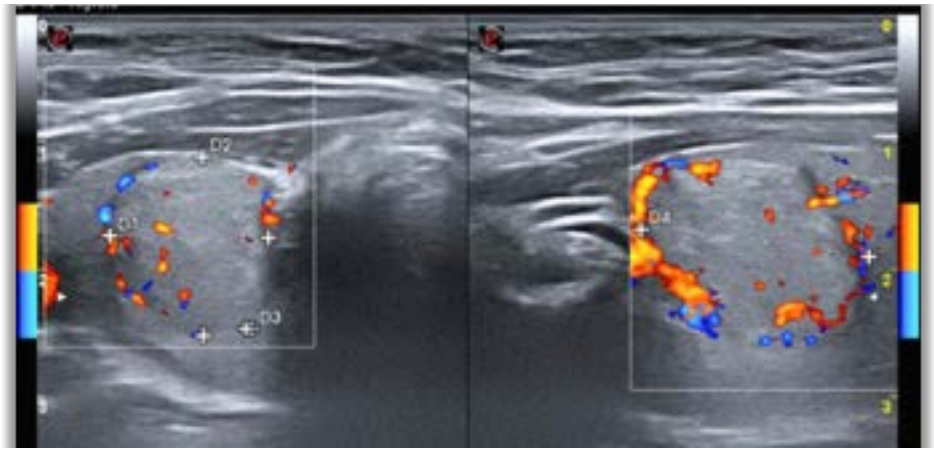

Figure 3. Ultrasound presentation of the low-risk nodule in the right thyroid lobe.

Although thyroid cancer usually has one or more of the following high-risk features, according to EU-TIRADS[1] (markedly hypoechoic structure, microcalcifications, blurred margins, irregular shape), there is a 2–4% risk for malignancy for isoechoic nodules. A possibility of occult thyroid cancer was also discussed (evident metastases with unknown position of the primary thyroid carcinoma). Lymphoma was also considered; however, the patient did not report any B symptoms (weight loss, night sweats, fever). No significant changes in the complete blood count were noted, although these are not mandatory for the diagnosis. Metastases from head and neck malignancies are not uncommon and smoking is a notable risk factor, as it is in most carcinomas. Finally, dissemination from a distant unknown primary site was also discussed. Although rare, cases of metastases to cervical lymph nodes from pulmonary, esophageal, gastric, pancreatic, ovarian and breast cancer have been reported[2,3].

A fine-needle aspiration biopsy (FNA) of lymph nodes in levels IV bilaterally was performed and the cytological interpretation confirmed malignancy. Wash-out analysis for thyroglobulin (FNA-Tg) and calcitonin (FNA-Ctn) was performed to rule out differentiated and medullary thyroid cancer. Interestingly, FNA-Tg from the lymph node on the left was markedly elevated (> 500 ng/ml), while on the right side it was negative (0.025 ng/ml). FNA-Ctn was negative in both lymph nodes. A repeat biopsy was performed with negative values of FNA-Tg from the lymph node on the left (0.244 ng/ml). The initial high value could be explained by contamination from the

adjacent thyroid tissue, which normally produces thyroglobulin. Multiple serum tumor markers were assessed, elevation only of CEA was noted (Table 1).

A chest CT scan revealed multiple lesions in both lungs, suspicious for malignancy. (Fig. 4)

Bronchoscopy with brush biopsy was performed. The histological material was positive for TTF-1 and negative for p40 and CD56, confirming the diagnosis of poorly differentiated adenocarcinoma of the lung. EGFR gene mutation (exon 19 deletion) was confirmed and the patient was started on osimertinib.

Lung cancer is the leading cause of cancer mortality, representing more than one-quarter of all cancer deaths in 2016[4]. The poorly differentiated lung adenocarcinoma usually has an unfavorable prognosis. Nevertheless, the discovery of oncogenic drivers, such as the epidermal growth factor receptor (*EGFR*) mutations and the development of specific molecularly targeted agents, has radically changed the therapeutic landscape for affected patients[5].

Rapid shrinkage of the cervical lymph nodes was noted two months after the initiation of treatment. Besides a transient epistaxis, the patient did not report any other signs of intolerance towards the targeted therapy.

Table 1. Tumor markers, complete blood count, thyroid function tests and biochemistry

TSH – 1,442 mU/l (0,38-5,33)	WBC – 7.17 x10^9/L (3,5-10,5)	CEA - 24,25 ng/ml (0-3)
fT4 – 9,38 pmol/l (7,86-14,41)	RBC - 4,84 x10^{12}/L (4-5,5)	CA 19-9 - 12,7 U/mL (0-37)
HbA1c – 7,8%	HGB - 135 g/l (140-180)	CA 72-4 - 2,44 U/ml (0-6)
Креатинин – 83 µmol/l (42-110)	PLT – 417 x10^9/L (140-180)	PIVKA II - 10.24 mAU/ml (0-40)
ASAT – 23 U/L (5-40)		CA 125 - 5.6 U/ml (0-35)
		CA 15-3 – 17.5 U/mL (0-35)

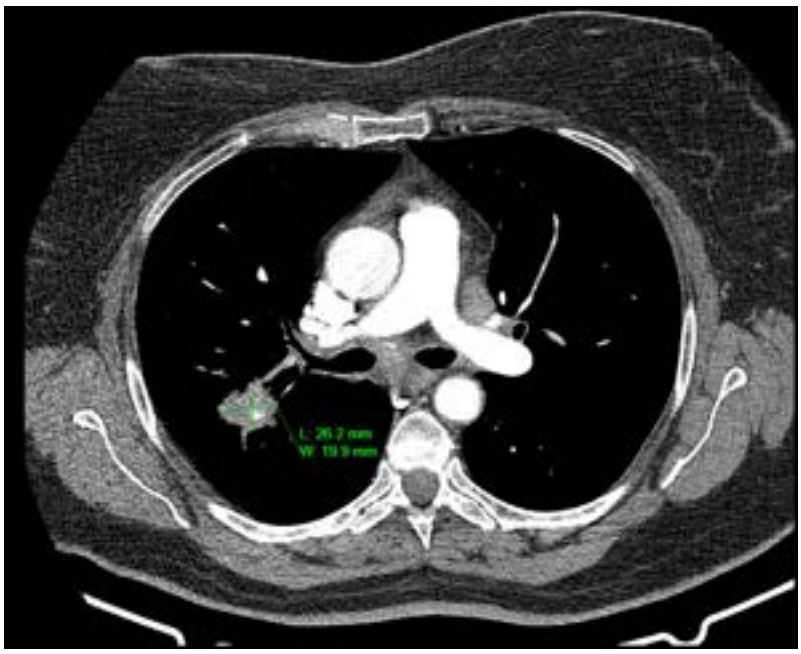

Figure 4. Chest CT scan revealing a formation in the right lung

Conclusion

Distant metastases to the cervical lymph nodes from lung cancer are uncommon. Nevertheless, it is crucial to bear in mind the possibility of such peculiarities in order to broaden the differential-diagnostic plan and reach the correct medical conclusion in a timely manner, which in cases of highly-aggressive tumors can be life-saving.

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Diagnosis and treatment of heart failure with preserved ejection fraction

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Introduction: Heart Failure with preserved Ejection Fraction (HFpEF) represents a major diagnostic and therapeutic challenge, accounting for nearly 50% of all heart failure cases. It is characterized by symptoms and signs of congestion despite preserved left ventricular ejection fraction (LVEF ≥ 50%) and is strongly associated with multiple cardiovascular and non-cardiovascular comorbidities. **Aim:** This review aims to summarize current diagnostic algorithms (HFA-PEFF, H2FPEF) and to outline contemporary management strategies for HFpEF. **Materials and methods:** A structured review of current ESC and AHA guidelines, along with key clinical trials including EMPEROR-Preserved and DELIVER, was performed, focusing on diagnostic approaches and evidence-based treatment options. **Results and discussion:** Diagnosis requires a multiparametric approach integrating clinical evaluation, echocardiographic assessment of diastolic dysfunction and structural remodeling, and natriuretic peptide measurement, with careful interpretation in the presence of chronic kidney disease. Pathophysiologically, HFpEF is driven by systemic inflammation, endothelial dysfunction, myocardial stiffness, and comorbidity-related myocardial remodeling. Chronic kidney disease, hypertension, obesity, and atrial fibrillation contribute substantially to disease progression and must be actively managed. Recent trials have demonstrated that sodium–glucose cotransporter 2 (SGLT2) inhibitors reduce heart failure hospitalizations across a broad HFpEF population, complementing comprehensive risk factor control and symptom-directed therapy. **Conclusion:** HFpEF management requires early and accurate diagnosis combined with an integrated therapeutic strategy targeting comorbidities, hemodynamic abnormalities, and disease-modifying pharmacotherapy. Recent advances, including SGLT2 inhibitors, represent an important component of contemporary treatment but should be considered within a broader multimodal approach.

Keywords: *HFpEF, SGLT2 inhibitors, Diastolic Dysfunction, Echocardiography, NT-proBNP*

Introduction

Heart failure with preserved ejection fraction (HFpEF) has emerged as one of the most significant challenges in contemporary cardiovascular medicine. Over the past two decades, its prevalence has increased steadily, and HFpEF currently accounts for approximately one-half of all heart failure cases. This trend is largely attributable to population ageing and the growing burden of cardiometabolic disorders. Despite substantial advances in diagnostic and therapeutic strategies, HFpEF remains associated with frequent hospitalizations, impaired quality of life, and an adverse prognosis comparable to that observed in patients with heart failure with reduced ejection fraction (HFrEF) [1, 2].

Unlike HFrEF, in which impaired left ventricular systolic function represents the principal pathophysiological mechanism, HFpEF is a heterogeneous clinical syndrome characterized by a complex interplay of myocardial, vascular, renal, and systemic factors. The traditional concept that HFpEF is primarily a consequence of diastolic dysfunction has gradually evolved into a broader paradigm recognizing the contribution of chronic inflammation, endothelial dysfunction, coronary microvascular abnormalities, myocardial fibrosis, and multimorbidity to adverse cardiovascular remodeling [3, 4].

Clinical presentation is frequently nonspecific, with symptoms such as exertional dyspnea, exercise intolerance, and fatigue overlapping with a wide range of cardiovascular and non-cardiovascular conditions. Consequently, structured diagnostic algorithms, including the HFA-PEFF and H2FPEF scores, have been developed to

integrate clinical, laboratory, and echocardiographic parameters, thereby improving diagnostic accuracy and facilitating a more standardized diagnostic approach [5–7].

Until recently, management of HFpEF was largely focused on symptom control and optimization of comorbid conditions, as most pharmacological therapies proven effective in HFrEF failed to demonstrate meaningful prognostic benefit in patients with preserved ejection fraction. However, the publication of the EMPEROR-Preserved and DELIVER trials marked a major turning point in the field by demonstrating, for the first time, a significant reduction in heart failure hospitalizations with sodium–glucose cotransporter 2 inhibitors (SGLT2i) across a broad HFpEF population [8, 9].

Growing recognition of the marked phenotypic heterogeneity among patients with HFpEF has further stimulated the development of personalized approaches to disease management. Emerging evidence suggests that distinct clinical phenotypes may exhibit different pathophysiological mechanisms, clinical trajectories, and therapeutic responses. Consequently, phenotypic profiling has become a central component of contemporary HFpEF research and is increasingly regarded as a cornerstone of future diagnostic and therapeutic strategies [10–12].

Aim

The aim of this review is to summarize contemporary concepts regarding the pathophysiology, diagnosis, and management of HFpEF, with particular emphasis on the HFA-PEFF and H2FPEF diagnostic algorithms, the role of biomarkers and phenotypic profiling, and emerging therapeutic strategies, including SGLT2 inhibitors and non-steroidal mineralocorticoid receptor antagonists.

Methods

A review of the current literature on HFpEF was conducted. Relevant consensus statements from the European Society of Cardiology (ESC), scientific statements from the Heart Failure Association (HFA), and key randomized clinical trials and observational studies were analyzed. Particular attention was given to diagnostic algorithms, biomarker-guided assessment, phenotypic classification, and contemporary pharmacological treatment strategies.

Results

Pathophysiological mechanisms of HFpEF

The development of HFpEF is driven by a complex network of interconnected pathophysiological processes involving not only the myocardium but also the vascular system, kidneys, lungs, skeletal muscle, and metabolic regulatory pathways. This complexity largely explains the marked clinical heterogeneity of the syndrome and the historically limited success of therapeutic interventions. Contemporary concepts increasingly recognize HFpEF as a systemic disorder in which cardiac dysfunction represents the final manifestation of multiple interacting comorbid conditions and biological mechanisms [3, 4].

Diastolic dysfunction remains a central component of HFpEF pathogenesis. Increased left ventricular stiffness and impaired myocardial relaxation result in elevated filling pressures, often evident during the early stages of the disease. Consequently, even modest physical exertion may provoke a substantial increase in pulmonary venous pressure, leading to exertional dyspnea and reduced exercise tolerance. These abnormalities are frequently accompanied by concentric left ventricular hypertrophy, increased myocardial mass, and left atrial enlargement [13, 14].

In recent years, particular attention has been directed toward the role of chronic low-grade inflammation. Hypertension, obesity, diabetes mellitus, and chronic kidney disease promote the production of pro-inflammatory mediators, including tumor necrosis factor-α, interleukin-6, and other cytokines. These processes contribute to endothelial dysfunction, reduced nitric oxide bioavailability, and impairment of the cyclic guanosine monophosphate–protein kinase G signaling pathway. The downstream consequences include increased myocardial stiffness, interstitial fibrosis, and progressive structural remodeling of the myocardium [3, 11].

Obesity is increasingly recognized as one of the most important drivers of contemporary HFpEF epidemiology. Beyond increasing circulating blood volume and hemodynamic load, adipose tissue functions as an active endocrine organ that secretes numerous pro-inflammatory and pro-fibrotic mediators. These mechanisms promote myocardial hypertrophy, impair cardiomyocyte energy homeostasis, and accelerate the progression of diastolic dysfunction. Consequently, obesity is increasingly considered not merely a risk factor but a distinct HFpEF phenotype characterized by unique pathophysiological features and clinical behavior [15].

Chronic kidney disease also plays a pivotal role in HFpEF pathogenesis. Cardiorenal interactions contribute to sodium and water retention, neurohormonal activation, vascular remodeling, and systemic inflammation. Renal dysfunction is associated not only with an increased risk of developing HFpEF but also with poorer outcomes after diagnosis. Recent evidence highlights the cardiorenal axis as a promising therapeutic target in this patient population [16].

Diagnostic approach to HFpEF

The diagnosis of HFpEF remains one of the greatest challenges in contemporary cardiovascular practice. Unlike HFrEF, where reduced left ventricular ejection fraction provides a relatively objective diagnostic criterion, HFpEF requires integration of clinical findings, laboratory data, and multimodality imaging parameters [5, 17].

According to the current ESC definition, HFpEF is characterized by symptoms and signs of heart failure, a left ventricular ejection fraction ≥ 50%, elevated natriuretic peptide concentrations, and objective evidence of structural and/or functional cardiac abnormalities. Nevertheless, a substantial proportion of patients – particularly those with obesity – may present with normal natriuretic peptide levels despite clinically significant disease, thereby complicating diagnostic assessment [1, 4].

To address these challenges, the Heart Failure Association of the ESC proposed the HFA-PEFF diagnostic algorithm in 2019. This structured approach consists of four sequential steps: pre-test clinical evaluation, echocardiographic and biomarker scoring, functional testing, and identification of the underlying etiology. Particular emphasis is placed on diastolic function parameters, left atrial volume index, left ventricular mass index, global longitudinal strain, and natriuretic peptide concentrations [5].

An alternative diagnostic tool is the H2FPEF score, which was developed using readily available clinical and echocardiographic variables. The score incorporates six parameters: obesity, treatment with two or more antihypertensive medications, atrial fibrillation, pulmonary hypertension, age greater than 60 years, and elevated E/e′ ratio. A major advantage of this model is its simplicity and applicability in routine clinical

practice without requiring advanced echocardiographic assessment [6, 18].

Comparative studies evaluating HFA-PEFF and H2FPEF have demonstrated significant discrepancies in patient classification. Yoon et al. reported limited concordance between the two diagnostic algorithms and substantial variation in HFpEF prevalence depending on the scoring system applied. These findings underscore the importance of integrating multiple diagnostic strategies rather than relying exclusively on a single algorithm [17].

Particularly challenging are patients with concomitant atrial fibrillation. Data from Ariyaratnam et al. suggest that both HFA-PEFF and H2FPEF demonstrate only moderate diagnostic accuracy in this population when compared with invasive hemodynamic assessment, which remains the reference standard. Consequently, diagnostic interpretation in patients with atrial fibrillation requires particular caution and often additional testing [18].

Role of biomarkers and echocardiography in the diagnosis of HFpEF

Given the marked clinical and pathophysiological heterogeneity of HFpEF, no single diagnostic parameter possesses sufficient sensitivity and specificity to independently confirm or exclude the diagnosis. Consequently, contemporary diagnostic strategies rely on the combined use of biomarkers, echocardiographic parameters, and functional testing. This multimodal approach forms the foundation of both the HFA-PEFF and H2FPEF diagnostic algorithms [1, 5].

Natriuretic peptides, particularly B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), remain the most widely utilized biomarkers in heart failure. Elevated concentrations reflect increased ventricular wall stress and elevated filling pressures. Although levels are generally lower in HFpEF than in HFrEF, increased natriuretic peptide concentrations are consistently associated with adverse clinical outcomes, including a higher risk of hospitalization and cardiovascular mortality [11].

However, important limitations must be considered when interpreting natriuretic peptide levels in HFpEF. Patients with obesity frequently exhibit lower-than-expected BNP and NT-proBNP concentrations despite clinically significant disease, potentially leading to underdiagnosis. Conversely, chronic kidney disease may result in elevated natriuretic peptide levels independent of heart failure severity. Therefore, these biomarkers should be interpreted within the broader clinical context rather than being used as isolated diagnostic indicators [1, 11, 15].

Recent advances have highlighted the potential value of novel biomarkers reflecting distinct biological pathways involved in HFpEF pathogenesis. High-sensitivity cardiac troponins, markers of fibrosis such as galectin-3 and soluble ST2, as well as proteomic and genomic biomarkers, have emerged as promising tools for risk stratification and biological phenotyping. These biomarkers provide additional insights into myocardial injury, inflammation, fibrosis, and renal dysfunction, thereby contributing to a more comprehensive understanding of disease heterogeneity [11].

Echocardiography remains the cornerstone imaging modality for evaluating patients with suspected HFpEF. Beyond confirming preserved left ventricular ejection fraction, echocardiography provides essential information regarding diastolic function, structural remodeling, and hemodynamic consequences of the disease. Frequently assessed parameters include the E/e′ ratio, left

atrial volume index, left ventricular mass index, and tricuspid regurgitation velocity [5, 13].

Particular attention has recently been directed toward the assessment of global longitudinal strain (GLS). Despite preserved ejection fraction, many patients with HFpEF exhibit subclinical impairment of longitudinal systolic function that can be detected using myocardial deformation imaging. Reduced GLS has been associated with adverse outcomes and may facilitate earlier identification of myocardial dysfunction before overt deterioration becomes apparent on conventional echocardiographic assessment [5].

In diagnostically challenging cases, stress echocardiography and invasive hemodynamic testing play an increasingly important role. A subset of patients demonstrates normal filling pressures at rest but develops significant elevations during exercise. In such situations, measurement of pulmonary capillary wedge pressure during exertion remains the gold standard for confirming the diagnosis of HFpEF and distinguishing it from alternative causes of exertional dyspnea [6, 19].

Phenotyping and personalized medicine in HFpEF

One of the most important conceptual advances in HFpEF research over the past decade has been the transition from viewing HFpEF as a single disease entity to recognizing it as a heterogeneous syndrome composed of multiple clinical phenotypes. The repeated failure of several large-scale clinical trials to demonstrate universal therapeutic benefit has emphasized the limitations of a “one-size-fits-all” approach and highlighted the biological diversity underlying the syndrome [4, 15].

Modern phenotyping strategies integrate extensive clinical, laboratory, imaging, and hemodynamic data to identify groups of patients with shared characteristics and disease mechanisms. This approach, commonly referred to as phenomapping, enables a more precise characterization of HFpEF subtypes and may facilitate the development of individualized treatment strategies [14, 20].

One of the landmark studies in this field was conducted by Cohen and colleagues, who identified three major phenogroups among patients with HFpEF. The first group consisted of relatively younger individuals with fewer structural abnormalities and a comparatively favorable prognosis. The second phenotype was characterized by a high prevalence of atrial fibrillation, left atrial enlargement, and vascular stiffness. The third phenotype included patients with obesity, diabetes mellitus, chronic kidney disease, and pronounced systemic inflammation, representing the subgroup with the highest risk of adverse cardiovascular outcomes [15].

Accumulating evidence suggests that these phenotypes differ not only in clinical presentation and prognosis but also in therapeutic responsiveness. Analyses from the TOPCAT trial indicated that patients belonging to the high-risk metabolic-inflammatory phenotype derived greater benefit from spironolactone therapy than other patient groups, further supporting the concept of phenotype-guided treatment [15].

The 2023 Heart Failure Association Scientific Statement emphasized the importance of phenotypic profiling as a framework for individualized therapeutic decision-making. The proposed classification incorporates dominant clinical features such as obesity, atrial fibrillation, hypertension, chronic kidney disease, and pulmonary hypertension. Such an approach has the potential to improve both diagnostic precision and treatment effectiveness while facilitating a more personalized model of care [12].

In parallel, artificial intelligence (AI) and machine learning technologies are increasingly being explored as tools for enhancing HFpEF diagnosis and management. Advanced computational algorithms are capable of identifying complex patterns within multidimensional clinical datasets, thereby improving diagnostic classification, prognostic assessment, and therapeutic stratification. Although these technologies have not yet been widely adopted in routine clinical practice, they are expected to play an increasingly important role in the evolution of precision medicine for HFpEF [14, 20].

Contemporary management of HFpEF

For decades, therapeutic options for HFpEF remained limited, and most pharmacological agents that demonstrated substantial benefits in HFrEF failed to improve outcomes in patients with preserved ejection fraction. However, the growing understanding of HFpEF pathophysiology and the emergence of novel therapeutic targets have significantly transformed the treatment landscape. Contemporary management is increasingly based on evidence-driven interventions combined with comprehensive control of comorbid conditions and individualized patient care [1, 4].

Current guidelines emphasize three fundamental principles in the management of HFpEF: relief of congestion and symptoms, optimal treatment of associated comorbidities, and implementation of therapies with proven prognostic benefit. Given the considerable heterogeneity of the syndrome, no universal therapeutic algorithm is suitable for all patients. Instead, treatment strategies should be tailored according to the dominant clinical phenotype and underlying pathophysiological mechanisms [12].

Diuretic therapy

Despite the absence of convincing evidence for mortality reduction, diuretics remain a cornerstone of symptomatic treatment in HFpEF. By reducing intravascular volume and lowering cardiac filling pressures, they provide effective relief of dyspnea, pulmonary congestion, and peripheral edema. Their use is particularly important during episodes of decompensation and volume overload.

Nevertheless, diuretic therapy requires careful monitoring, especially in elderly patients and individuals with chronic kidney disease. Excessive diuresis may precipitate hypotension, worsening renal function, and electrolyte disturbances. Consequently, treatment should be individualized according to volume status, renal function, and clinical response [4].

SGLT2 inhibitors: a new standard of care in HFpEF

The most important therapeutic breakthrough in HFpEF during the past decade has been the introduction of SGLT2 inhibitors. These agents represent the first drug class to demonstrate consistent clinical benefit across a broad HFpEF population regardless of diabetes status.

The EMPEROR-Preserved trial enrolled 5,988 patients with heart failure and an ejection fraction greater than 40%, randomizing them to empagliflozin or placebo. After a median follow-up of 26.2 months, empagliflozin reduced the relative risk of the composite endpoint of cardiovascular death or hospitalization for heart failure by 21% (HR 0.79; 95% CI 0.69–0.90). This benefit was driven primarily by a significant reduction in heart failure hospitalizations [8].

These findings were subsequently confirmed by the DELIVER trial, which included 6,263 patients with mildly reduced or preserved ejection fraction. Treatment with dapagliflozin significantly reduced the risk of worsening heart failure or cardiovascular death (HR 0.82; 95% CI 0.73–0.92).

Importantly, the therapeutic benefit was observed irrespective of diabetic status and across the entire spectrum of ejection fraction represented in the study population [9].

Although the precise mechanisms underlying the beneficial effects of SGLT2 inhibitors remain incompletely understood, several pathways have been proposed. Potential mechanisms include improvements in myocardial energetics, reductions in systemic inflammation and oxidative stress, modulation of neurohormonal activation, and favorable effects on renal function. Emerging experimental evidence further suggests that dapagliflozin may improve mitochondrial function through activation of the SIRT1/PGC-1α signaling pathway, thereby contributing to its cardioprotective effects [8, 9].

Given the consistency of evidence across multiple clinical trials, SGLT2 inhibitors are now considered a foundational component of contemporary HFpEF management and have been incorporated into international guideline recommendations as first-line disease-modifying therapy [1, 2].

Mineralocorticoid receptor antagonists and finerenone

Mineralocorticoid receptor activation contributes substantially to myocardial fibrosis, inflammation, endothelial dysfunction, and adverse cardiovascular remodeling. Consequently, mineralocorticoid receptor antagonists (MRAs) have long been investigated as potential therapeutic agents in HFpEF.

Although the TOPCAT trial yielded mixed overall results, post hoc analyses suggested clinically meaningful benefits in selected patient subgroups, particularly those characterized by obesity, metabolic dysfunction, and heightened systemic inflammation. These observations reinforced the concept that therapeutic efficacy in HFpEF may depend on underlying phenotype rather than solely on ejection fraction [15].

More recently, attention has shifted toward finerenone, a non-steroidal mineralocorticoid receptor antagonist with a more favorable safety profile than traditional MRAs. The FINEARTS-HF trial demonstrated a significant reduction in the composite risk of worsening heart failure events and cardiovascular death among patients with HFmrEF and HFpEF treated with finerenone [16].

In addition to its direct cardiovascular effects, finerenone exerts protective actions on renal function, an important consideration given the high prevalence of chronic kidney disease among patients with HFpEF. These findings position finerenone as one of the most promising emerging therapeutic options and suggest a potential future role alongside SGLT2 inhibitors in comprehensive disease management [16].

Future therapeutic perspectives

Despite substantial progress, HFpEF remains a condition with limited disease-modifying treatment options compared with HFrEF. Ongoing research is focused on developing therapies that target specific biological pathways involved in inflammation, myocardial fibrosis, metabolic dysfunction, endothelial injury, and microvascular disease.

At the same time, advances in phenotypic profiling and artificial intelligence are creating new opportunities for precision medicine. Future therapeutic strategies may increasingly rely on individualized biological and clinical characteristics rather than conventional classifications based solely on ejection fraction. Such an approach could improve patient selection for specific therapies and maximize treatment effectiveness while minimizing unnecessary interventions [12, 14, 20].

Conclusion

Optimal management of HFpEF requires early and accurate diagnosis, rigorous control of comorbid conditions, implementation of evidence-based pharmacological therapies, and a personalized treatment strategy guided by the individual patient phenotype. A deeper understanding of the biological heterogeneity of HFpEF will likely be essential for future advances in diagnosis, treatment, and prognosis, ultimately improving outcomes for this growing patient population.

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Brief review of interstitial lung disorders with a focus on the MDT approach in diagnosis and management

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Interstitial lung diseases (ILDs) are a group of disorders affecting the alveolar wall and causing diffuse scarring or fibrosis. The group includes more than 150 diffuse parenchymal lung diseases (idiopathic, connective tissue disorders-related, caused by environmental factors or iatrogenic). The clinical presentation of ILDs is usually nonspecific, with overlapping pathological and radiological features. Routine investigations are usually insufficient for the definitive diagnosis. As many ILDs are rare, the diagnosis presents a challenge to medical professionals and requires a coordinated effort by various specialists. The diagnosis is a multistep process, including initial assessment by pulmonologist with detailed functional testing. High-resolution computed tomography (HRCT) is essential in this process, helping to distinguish patterns such as usual interstitial pneumonia (UIP) from other morphologies. Imaging alone could be inconclusive and distinction between different subtypes requires invasive biopsy procedures and histology. Exclusion of connective tissue disorders makes complex antibody panels testing necessary. Given this complexity, a multidisciplinary diagnostic approach has become the established standard. This collaborative model synthesises expertise from pulmonology, thoracic radiology, and pathology as well as with rheumatology when indicated to correlate clinical, radiological, and histopathological findings. This review focuses on the application of this multidisciplinary framework to key entities, including idiopathic pulmonary fibrosis (IPF), connective tissue disease-associated ILD, and hypersensitivity pneumonitis. The consistent use of such a structured, standardised and collaborative approach is fundamental to achieving precise diagnoses and improving patient outcomes in ILD care.

Keywords: *ILDs, MDT, diagnosis*

Definition, epidemiology & burden of interstitial lung disease

Interstitial lung diseases (ILDs) are a heterogeneous group of disorders characterised by inflammation and progressive fibrosis of the lungs. Initially, the interstitial lung space becomes infiltrated with inflammatory cells, followed by fibrotic tissue. This leads to a derangement of the alveolar capillary unit and impaired gas exchange [1].

Epidemiology

Due to the diversity of ILD conditions, paucity of guidance and updates to diagnostic criteria over time, it has been challenging to precisely determine ILD incidence and prevalence [2]. A recent study using Global Burden of Disease data indicated that, the global ILD incidence in the past 10 years has risen by 51% (313.2 cases in 1990 to 207.2 per 1 00 000 cases in 2019) [3]. At present, the majority of studies report a worldwide prevalence of IPF ranging from 7 to 42 per 100 000 persons across different case definitions [2]. Regarding non IPF ILDs, most data cover the incidence and prevalence of those associated with autoimmune disorders. The majority of studies show a significant heterogeneity in the reported prevalence of ILD associated with connective tissue disorders (as systemic sclerosis – SSc, rheumatoid arthritis – RA and Sjogren's syndrome. The prevalence of ILD in SSc ranged from 26.1% (Australia, 2015) [4] to 88.1% (India, 2013) [5]. Similarly, Sjogren's ILD ranged from 1% (Sweden, 2011) [6] to 87.8% (Saudi Arabia, 2021) [2]. Regarding occupational ILDs, they are more common in low-income and middle-income countries in Asia and South America (42.8% are in Asia) [2]. Generally, most researchers in this area stress the lack of uniformity in the published estimates of incidence and prevalence of ILD conditions and as a result, the exact

burdens of these diseases is at present difficult to assess [2].

Economic burden of ILD

ILDs represent a significant source of premature mortality and disability-adjusted life years (DALYs) lost [7]. Global ILD costs can vary, with a reported median direct annual cost of US$ 32,834 per patient [7]. Hospitalisations have been the most significant expense (55% of direct costs), followed by outpatient care (22%) and pharmacotherapy (18%). This distribution remains prominent also in recent findings – a German study reported that inpatient care accounted for 69% of total costs (€7,687 per patient/year) [8].

Classification of ILDs (including Idiopathic Pulmonary Fibrosis and Hypersensitivity Pneumonitis) is presented in Figure 1 [9].

Clinical presentation & initial evaluation

Interstitial Lung Diseases have common lung symptoms, however, the presence of specific extra-pulmonary signs is also important for the multidisciplinary team to differentiate between idiopathic, secondary and systemic origins.

Interstitial lung disease patients typically present with progressive exertional dyspnea and persistent dry cough. However, these symptoms can vary across the spectrum of diseases – for instance, the acute onset of fever alongside acute respiratory distress may indicate acute interstitial pneumonia (AIP) or drug induced injury. Wheezing is uncommon in ILDs, but could be reported by patients with Hypersensitivity Pneumonitis, Chronic Eosinophilic Pneumonia or Eosinophilic Granulomatosis with Polyangiitis also known as Churg-Strauss Syndrome. Likewise, hemoptysis is uncommon but important, suggesting diffuse alveolar haemorrhage, (Goodpasture Syndrome). Recurrent sinusitis may suggest Wegner's Granulomatosis and Churg Strauss Syndrome. Sicca symptoms (dry eyes and mouth) and uveitis are suggestive of Sarcoidosis or Behcet's Disease. Pleuritic chest pain could be reported by patients with Systemic Lupus Erythematosus and Sarcoidosis. In collagen vascular diseases, especially Rheumatoid Arthritis and Systemic Lupus Erythematosus, as well as drug-related ILDs pleurisy can occur.

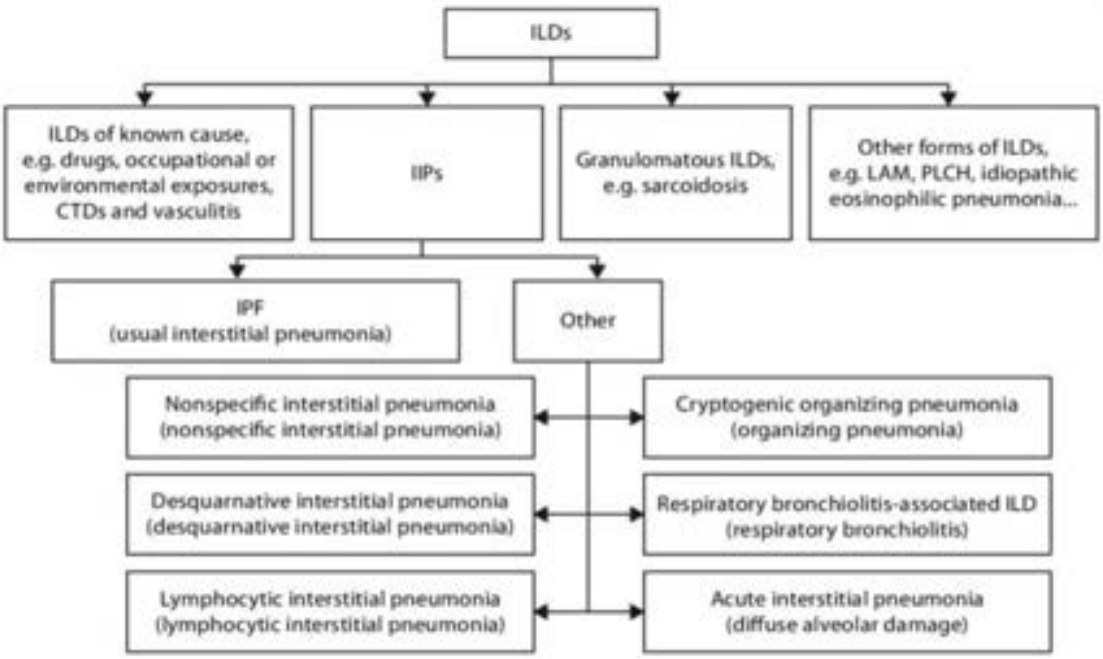

Figure 1. Classification of ILDs.

Lung auscultation in Interstitial Lung Diseases (ILDs) characteristically reveals **fine, dry, bibasilar inspiratory crackles,** often described as "Velcro-like". However, in granulomatous ILDs (e.g hypersensitivity pneumonitis, sarcoidosis, silicosis), the crackles are less prominent.

Digital clubbing could be observed in Idiopathic Pulmonary Fibrosis (IPF). Wheezing could be auscultated in hypersensitivity pneumonitis. Later in the course of ILDs, signs of pulmonary hypertension and cor pulmonale could be seen [10].

ILDs mechanisms and pathology

Approximately 65% of patients with ILDs are victims of a process for which no etiological agent has been identified. For the remaining, a causative agent has been identified – those include: diseases caused by inhaled inorganic dusts, hypersensitivity pneumonitis, drug and radiation induced ILDs. Regardless of the vast number of ILDs, it isgenerally believed that they share similar pathogenic mechanisms. The initial stimulus is either a toxin or an allergen. Genetic factors also play a role either as a primary determinant or a modifier of patients' response to a potentially injurious exposure [11]. Typically, ILDs, have two major pathologic components: inflammation in the alveolar wall and in the alveolar spaces (alveolitis) and a scarring or fibrotic process. The general presumption is that inflammation is the primary process and fibrosis follows as a secondary feature. IPF is a an exception, in which, alveolar injury is the primary process and fibrosis comes as an abnormal repair to the injury [12]. In alveolitis, various inflammatory cells (macrophages, lymphocytes, neutrophils, eosinophils and plasma cells) infiltrate the alveolar wall. They release mediators, toxic oxygen radicals, proteolytic enzymes etc. thus propagating the inflammation. Some of these mediators are capable of recruiting and stimulating the fibroblasts to produce new connective tissue. The final result is degradation of the lung parenchyma and connective tissue matrix and replacement by abnormal fibrous tissue (Fig. 2).

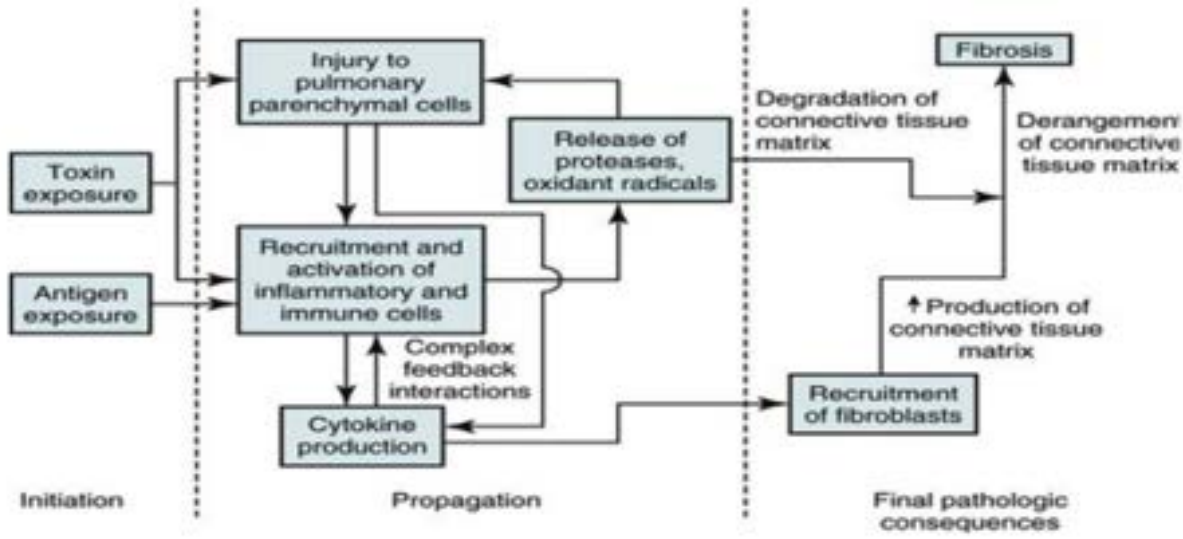

Figure 2. Pathogenesis of ILDs. Overview of Diffuse Parenchymal Lung Diseases – Clinical Gate

Pathologically, ILDs present with a number of distinct patterns: 1. UIP (usual interstitial pneumonia) – characterized by patchy areas of interstitial inflammation and collections of proliferating fibroblasts. Fibrosis is often associated with honeycombing which represents cystic air spaces that result from the retraction of the surrounding fibrous tissue. The pattern is typical for IPF, however, it could be seen also in ILDs due to dust exposure, those related to drugs and in lung involvement in rheumatic diseases. 2. NSIP (non-specific interstitial pneumonia) is characterized by mono-nuclear cell infiltration in the alveolar wall and variable uniform fibrosis, usually less apparent compared to UIP. It is seen as an idiopathic form or is associated with rheumatic diseases [13]. 3. Smoking related ILDs present pathologically with pigmented macrophages within the lumen of respiratory bronchioles, accompanied by infiltrates of lymphocytes and macrophages. 4. In the acute and subacute interstitial pneumonias, there is organizing fibrosis, accompanied by inflammation in the small airways. 5. End stage ILDs are typical with the presence of significant fibrosis, resulting in areas of contraction and formation of cystic spaces.

Diagnosis

The diagnosis of ILDs requires a multidisciplinary approach combining clinical history, high-resolution computed tomography (HRCT), pulmonary function tests, and sometimes surgical biopsy. Initially, it is important for primary care physicians to properly evaluate usually middle aged patients, presenting with dry cough or progressive exertional dyspnea and bibasilar Velcro-crackles on auscultation. The chest X-ray these days plays a limited role in the diagnosis. Perhaps the most important advance in the past 20 years for the diagnosis of ILD has been HRCT [14]. The HRCT findings that are helpful in the diagnosis of ILD include the pattern of parenchymal abnormality (eg, consolidation, reticular pattern), the anatomic distribution (upper vs lower, central vs peripheral), and associated findings (eg, mediastinal lymphadenopathy) [14]. The process usually requires an experienced radiologist, specializing in the diagnosis of ILDs. Often, final diagnosis could be achieved based on CT findings alone. However, at times, microscopic evaluation is necessary. The specimens could be obtained by flexible bronchoscopy and trans-bronchial biopsy or WATS. WATS is often the favoured approach, as it can obtain larger tissue samples. Additionally, BAL could be helpful in identifying the cells populations in ILDs [15]. Therefore, a team approach is crucial for the diagnosis, including experienced respiratory specialists, thoracic surgeons and pathologists. Pulmonary function testing is also needed in the evaluation of patients with suspected ILD and should include spirometry, diffusing capacity for carbon monoxide, lung volumes, and oximetry (oxygen saturation at rest and with exercise). Pulmonary function testing in patients with ILDs typically reveals a restrictive pattern with reduced diffusing capacity. Different laboratory test are also necessary and an evaluation by a rheumatologist is important to diagnose CTD associated ILDs. Serological panels usually include: anti-cyclic citrullinated peptide antibodies and antinuclear antibodies to exclude RA and SLE. Antineutrophil cytoplasmic antibody and antiglomerular basement membrane antibody assays are helpful in the diagnosis of Wegener granulomatosis and Goodpasture syndrome [13].

Finally, multidisciplinary teams (MDT) involving the integrated collaboration of healthcare professionals, are increasingly used in clinical practice to diagnose and treat interstitial lung diseases [14]. The

ATS/ERS consensus statements and 2017 Fleischner Society white paper suggest that MDTs should, as a minimum, include a pulmonologist, a radiologist and a pathologist to integrate different clinical data before forming a final diagnosis of ILDs [4]. Moreover, the involvement of a rheumatologist is crucial in the assessment of CTD-ILDs.

Pharmacological management

Antifibrotic Therapy: For idiopathic pulmonary fibrosis (IPF) and other progressive fibrosing ILDs for example, fibrotic hypersensitivity pneumonitis, connective tissue disease-associated ILD, antifibrotic agents are the first line treatments. Nintedanib, a tyrosine kinase inhibitor that blocks multiple growth factor receptors including PDGF, FGF, and VEGF and pirfenidone, an anti-fibrotic and anti-inflammatory agent have both been shown to reduce forced vital capacity (FVC) decline by approximately 50% compared to a placebo, thereby slowing disease progression and potentially preserving quality of life for longer. The MDT should initiate these drugs in patients with progressive disease, regardless of baseline FVC, as early treatment equals greater long-term benefits. However, the MDT must also be responsible for active monitoring of common gastrointestinal side effects (diarrhoea, nausea, vomiting, dyspepsia) and liver enzyme elevations, particularly during the first three to six months of therapy [16]. ***Immunosuppressive therapy:*** in ILDs as sarcoidosis, nonspecific interstitial pneumonia (NSIP) and hypersensitivity pneumonitis, corticosteroids remain first-line therapy, often combined with steroid-sparing agents like mycophenolate mofetil, azathioprine, or rituximab for patients who cannot taper steroids without relapse or who develop significant steroid-related toxicity. The MDT, including a rheumatologist where appropriate, should establish a clear tapering schedule to avoid long-term adverse effects such as osteoporosis, diabetes, weight gain, and proximal myopathy [17]. ***Non-pharmacological management*** is essential for symptom control, preservation of quality of life, functional independence, and even survival. The MDT must expand beyond the core respiratory physician team to include physiotherapists, occupational therapists, dietitians, clinical psychologists, palliative care specialists, social workers, and specialist nurses [17]. An example of this are the pulmonary rehabilitation programs, typically individually based on the patient's functional status, lasting 8 to 12 weeks and including strength training and aerobic exercise with continuous oxygen saturation monitoring [18]. ***Oxygen therapy*** is initiated in patients with respiratory failure. The MDT plays a crucial role in prescribing the correct flow rate, selecting the appropriate delivery device like a nasal cannula, reservoir cannula, or trans-tracheal oxygen and arranging home oxygen assessment by a respiratory therapist or oxygen nurse. [19]. GERD is documented in most patients with ILDs and it is thought to play a role in the progression and prognosis of the disease with increased risk of micro aspirations of gastric content. This shows the importance of MDT management of GERD in ILD typically including proton pump inhibitors (e.g. omeprazole, esomeprazole, pantoprazole) or histamine 2 receptor antagonists as first line therapy taken 30 minutes before a meal [19]. The MDT should monitor whenever disease behaviour changes, including rapid progression (FVC decline > 10% in six months), acute exacerbations, declining performance status. Specific MDT responsibilities in long-term care include: looking into progression by excluding alternative causes (e.g., infection, heart failure, pulmonary embolism, medication non-adherence); adjusting and monitoring pharmacological therapy; escalating supportive care by increasing oxygen

flow rate, initiating palliative oxygen, referring to pulmonary rehabilitation or psychological support; referring for lung transplant evaluation when criteria are met [20] and transitioning to palliative care with formal advance care planning, including discussion of resuscitation status, preferred place of death, and initiation of an end-of-life breathlessness management plan. The MDT should ensure that these conversations occur before a crisis, ideally when the patient is clinically stable, and that they are documented clearly in the medical records and shared with primary care [21].

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Tuberculosis of the prostate – a case series

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Background: More than 140 years after the identification of Mycobacterium tuberculosis, TB remains an important public health problem. Annually, 10 million cases of active disease are registered worldwide with approximately 1.8 million deaths. Genitourinary tuberculosis (GUTB) is the third most common form of extra-pulmonary disease (20–40% of the cases). However, prostate tuberculosis is extremely rare with an incidence of 2.6 to 6.6% among all GUTB cases. The diagnosis is challenging due to its non-specific presentation, often mimicking advanced prostate cancer. **Case presentation:** The first case is a 72-year-old man complaining of difficulty of urination and suprapubic pain. The second is a 65-year-old man who presented with macroscopic hematuria and difficult urination. Digital rectal examination findings were suggestive of cancer. CBC, biochemistry, urine testing and urine culture were unremarkable. Both patients presented with elevated PSA and unremarkable findings on chest X-ray. Prostate biopsy was diagnostic as it revealed the typical tuberculosis caseating granulomas, with layers of lymphocytes, macrophages, Langhans giant cells, and epithelioid cells. **Conclusion:** Isolated prostate tuberculosis is rare and can mimic both prostatitis and cancer by its clinical presentation and digital rectal examination findings. Diagnosis is usually achieved by a biopsy and positive IGRA tests. We consider this publication useful in informing physicians of this rare presentation of tuberculosis, and hope that it can help lead to earlier diagnosis of the disease.

Keywords: *extrapulmonary tuberculosis, urogenital tuberculosis, tuberculosis of the prostate*

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis,* is a major global infectious disease. Predominantly it affects the lungs, and is one of the leading causes of mortality worldwide [1]. In 2024, an estimated 10.7 million people developed TB, resulting in approximately 1.23 million deaths, including 150,000 among individuals with HIV Infection [2]. TB may be classified as pulmonary or extrapulmonary, depending on the site of involvement [3]. Extrapulmonary tuberculosis accounts for approximately 20–25% of all TB cases, including organs such as the abdomen, genitourinary tract, bones and joints [4]. Genitourinary TB accounts for 20–40% of those cases, and prostate TB accounts for just 2.6% of the GU TB cases. Prostate tuberculosis is an uncommon form of genitourinary tuberculosis and is often detected incidentally following transurethral resection of the prostate [5]. The most common route of involvement is hematogenous spread, although descending infection and direct intracanalicular extension have also been described [5]. Our aim is to present two cases of tuberculosis of the prostate gland with no pulmonary involvement, in order to increase awareness of this rare manifestation.

Case presentation

The first case is that of a 72-year-old patient (RI), the second – that of a 65-year-old (HZ). Initially both of them attended Urology Clinics. Upon admission, RI had low grade fever of 2–3 weeks duration, urinary urgency, and pain on urination. HZ was afebrile and reported urinary retention, diffuse pain, and discomfort in the lower abdomen. Both had gross hematuria and elevated PSA levels. Digital rectal examination findings were that of enlarged, firm prostate gland, with irregular nodularity, which raised a high suspicion of malignancy. Neither of them reported respiratory symptoms. HZ had a relative diagnosed and treated for TB. In both patients, CBC was generally unremarkable. Biochemical tests showed mildly elevated inflammatory markers as CRP and

fibrinogen. The initial presentation resembled carcinoma of the prostate, therefore the next step was for the attending urologists to proceed with US guided transrectal prostate biopsy. Histological smears were consistent with TB inflammation: multiple TB granulomas with central caseous necrosis with giant Langhans type cells, abundant lymphocytes and plasma cells. As a TB diagnosis was confirmed, imaging of the lungs was performed in both patients. RI had a normal chest X-ray. A small pleural encapsulation was noted at the level above the carina on the right in HZ. In both cases, there were no signs of infiltrates in the lung parenchyma as well as no cavities or fibrotic changes and calcifications, indicating active or past lung TB.

Discussion

Despite the widespread nature of TB and the incidence of the disease, genitourinary TB remains a rare occurrence, and prostate TB is even rarer still. Tuberculosis can spread to the prostate through three pathways: hematogenous dissemination from the lungs, descending infection from another part of the genitourinary tract, or through sexual transmission. Isolated prostate tuberculosis spread through sexual contact is extremely rare. This route of transmission is still questionable and requires more investigation. We demonstrate two cases of isolated prostate tuberculosis as a primary manifestation of TB, without any pulmonary involvement.

Our first patient is a 65-year-old man who was initially admitted to the urology department with a 2–3 week history of fever. He received antibiotic treatment in the urology department and was discharged. One week later he was again hospitalized in urology due to symptoms of urinary retention, diffuse pain and discomfort in his lower abdomen, and macroscopic hematuria. The patient did not report any respiratory symptoms such as cough or sputum production, nor did he present systemic symptoms such as weight loss. On physical examination, the patient was in a generally preserved condition. He was conscious and oriented, with no signs of cyanosis or dyspnea. Pulmonary auscultation revealed clear breath sounds bilaterally. The cardiovascular system was stable, with a blood pressure of 140/80 mmHg. The abdomen was soft and non-tender, with no evidence of organomegaly or peripheral edema. He has a past medical history of carcinoma of the prostate and he had a past smoking history of 20 pack years. His laboratory results are shown in Table 1.

Table 1. Laboratory tests during hospitalization

Parameter (reference range)	**Value**
Hemoglobin (110–160 g/L)	134 g/L
RBC (3.5–5.5 $x10^{12}$/L)	4.56 $\times10^{12}$/L
WBC (3.5–10 $x10^{9}$/L)	6.41 $\times10^{9}$/L
Platelets (140–400$\times10^{9}$/L)	255 $\times10^{9}$/L
ESR (< 20 mm/hr)	20 mm/h
Creatinine (65.4 to 119.3 µmol/L)	75 µmol/L
Total protein (60 to 83 g/L)	76.1 g/L
AST (0–35)	29 U/L
ALT (4 to 36 U/L)	32.8 U/L
Total bilirubin (3 to 17 mmol/L)	6.6 mmol/L

Urinalysis showed no bilirubin and only mild leukocytes. PSA was slightly elevated, and HIV testing was negative. A digital rectal exam (DRE) was performed and the results were a prostate with enlarged, firm, and irregular nodularity. A digital rectal exam with such findings, along with elevated PSA, and the urinary symptoms can be a sign of prostate carcinoma. Therefore, the next step performed was a transurethral resection of the prostate, in order to obtain a biopsy. Histological examination revealed findings consistent with tuberculosis; multiple TB

granulomas with central caseous necrosis, giant Langhans type cells, abundant lymphocytes and plasma cells. Once diagnosis of TB was confirmed, X-ray of the lungs, sputum smear and culture, and Mantoux test were performed.

Figure 1. Plain thoraco-abdominal X-ray

Mantoux test was positive but the X-ray and sputum smear and culture were all negative for current or past TB infection. The final diagnosis was isolated tuberculosis of the GU system, specifically isolated prostate tuberculosis. The patient was subsequently admitted for treatment.

Our second patient is a 72-year-old man admitted to the urology department for progressive lower urinary tract symptoms, including worsening dysuria, urinary retention, and lower abdominal pain. His past medical history included arterial hypertension, left bundle branch block, and a renal cyst. He also reported a previous, unspecified pulmonary illness treated for approximately one month. He has a family history of tuberculosis (aunt suffered from and died from TB), and he has been a smoker for 40 years. His regular medication included torasemide, carvedilol, and duodart. On admission, the patient was in a moderately impaired general condition but was conscious, oriented, and afebrile. Respiratory status was stable with a respiratory rate of 16/min and clear lung auscultation. Cardiovascular examination revealed stable hemodynamics with a blood pressure of 120/80 mmHg. The abdomen was soft and non-tender, without hepatosplenomegaly or peripheral edema.

Table 2. Laboratory tests during hospitalization

Parameter (reference range)	Value
Hemoglobin (110–160 g/L)	129 g/L
RBC (3.5–5.5 x10^{12}/L)	4.50 ×10^{12}/L
WBC (3.5–10 x10^{9}/L)	8.27 ×10^{9}/L
Platelets (140–400×10^{9}/L)	196 ×10^{9}/L
ESR (< 20 mm/hr)	86 mm/h
Total protein (60 to 83 g/L)	71.1 g/L
Creatinine (65.4 to 119.3 µmol/L)	123 µmol/L
Urea (1.8 to 7.1 mmol/L)	8.3 mmol/L
Fibrinogen (2–4 g/L)	7.93 g/L
AST (0–35 U/L)	13.2 U/L
ALT (4 to 36 U/L)	6.4 U/L
Iron (13–27 micromol/L)	6.8 µmol/L

Inflammatory activity was markedly elevated, reflected by a significantly increased ESR and a strongly elevated fibrinogen level. Iron levels were reduced, suggesting a possible anemia of chronic disease pattern. Biochemical analysis showed mildly impaired renal function with elevated creatinine and urea. Initial evaluation in the urology department included a digital rectal exam which showed enlarged, firm, and irregular nodularity. Just as for our first patient, the DRE findings along with the laboratory results and the patient's symptoms point to carcinoma of the prostate. Prostate biopsy was performed and it revealed chronic granulomatous inflammation with caseous necrosis and glandular destruction. Again, further testing was performed. Mantoux test was positive, CT of the lungs was negative for any TB findings, and sputum testing was negative.

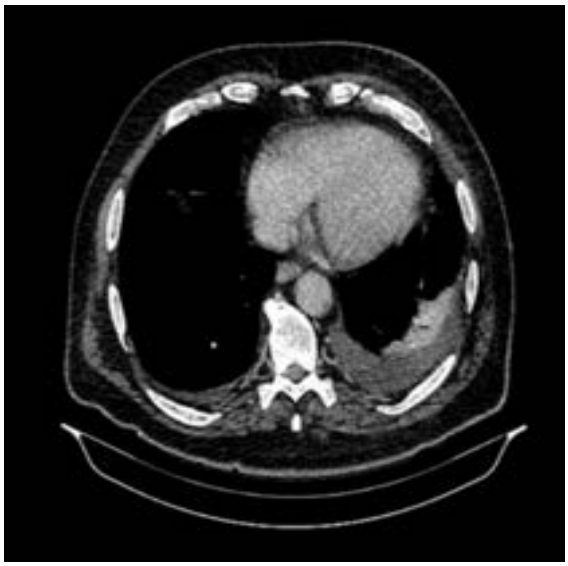

Figure 2. Contrast enhanced thoracic tomography

The final diagnosis was genitourinary tuberculosis, specifically isolated prostate tuberculosis.

Conclusion

Isolated prostate tuberculosis is rare and can mimic both prostatitis and cancer by its clinical presentation and digital rectal examination findings. Diagnosis is usually achieved by a biopsy and positive IGRA tests. We consider this publication useful in informing physicians of this rare presentation of tuberculosis, and hope that it can help lead to earlier diagnosis of the disease.

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From analgesia to overdose: the dual face of fentanyl in modern medicine and illicit drug use

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Introduction: Fentanyl is a potent synthetic opioid used as an analgesic and anesthetic. Opioids are a class of drugs that include both illegal drugs like heroin and prescription ones like morphine. Fentanyl is very effective in treating cancer pain, chronic pain and post-surgical conditions. The side effects include euphoria, sedation, respiratory depression and loss of consciousness. Even a small dose of fentanyl can be fatal. As an illicit drug, fentanyl is widely distributed, highly addictive and is linked to a high risk of fatal overdose. **Aim:** To investigate the dual role of fentanyl in medical practice and as an illicit drug, and to discuss contemporary guidelines for antidote use that can prevent lethal overdoses. **Materials and methods:** A literature-based review was conducted that summarizes the different uses of fentanyl and its antidotes in major medical databases. Observational studies, clinical and peer-reviewed articles published in English were considered. **Results and discussion:** Fentanyl is a potent opioid essential for treatment of severe pain but is also associated with high addictive potential and high overdose mortality. Illicit fentanyl has led to a significant rise in fatal overdoses. Naloxone, as an antidote, is a highly effective medication and wider access is crucial for individuals with addiction. **Conclusion:** Fentanyl has an important role in contemporary medicine but poses an enormous public health risk when used inappropriately. Reducing fentanyl related mortality requires new strategies like broad access to effective antidotes, recognition of the addiction in the early stages and safe medical use while strengthening prevention.
Keywords: *fentanyl, naloxone, opioid*

Introduction

Fentanyl is a synthetic opioid that plays an important role in modern pain management and anesthesia [1]. It was synthesized by Paul Janssen in 1960 [2]. His goal was to develop a more potent and rapid-acting analgesic than morphine [3]. Fentanyl belongs to the piperidine class of opioids and functions as an agonist of the μ-opioid receptor [3]. Due to its pharmacological properties, fentanyl is approximately 100 times more potent than morphine and 50 times more potent than heroin [3]. The high lipophilicity of fentanyl allows rapid penetration across the blood-brain barrier and rapid distribution to highly perfused tissues such as the brain, lungs, and heart [4]. These pharmacokinetic properties explain the rapid onset of analgesia and its effectiveness in surgical operations, intensive care units, and cancer pain management. The problem we face is that the same properties that make fentanyl highly effective also contribute to its high toxicity and abuse. In recent years, illicitly produced fentanyl and its analogues have become major contributors to the global opioid crisis and the increasing overdose deaths worldwide [3].

Aim

The purpose of our literature review is to systematize the knowledge related to the study of the dual role of fentanyl in modern medicine, as well as the risk of its use as an illicit drug. The article analyzes the pharmacological properties of fentanyl, which make it valuable for the treatment of severe pain. It also examines epidemiological data explaining how fentanyl has become a major problem in the emergence of the opioid crisis and the mechanisms of fentanyl toxicity and strategies for the prevention and treatment of overdose.

Methods

For the purpose of this article, a literature review of various scientific articles was conducted. Selected publications included pharmacological analyses of fentanyl, epidemiological reports examining the opioid crisis, fentanyl-related mortality, and current antidote therapy. The literature was analyzed to identify key pharmacological mechanisms of action of fentanyl, patterns of illicit use of fentanyl, and statistical trends related to opioid overdose deaths.

Results

Pharmacological basis of fentanyl analgesia

Fentanyl is characterized by high lipid solubility, which allows rapid penetration into the central nervous system and produces strong analgesic effects [4]. Due to its high potency, the analgesic effect can be achieved with very small doses compared to other opioids. The drug is metabolized in the liver and excreted in the urine as inactive metabolites, such as norfentanyl [4]. Redistribution of fentanyl from highly perfused organs to muscle and adipose tissue contributes to its prolonged elimination phase.

Mechanisms of fentanyl toxicity

The main life-threatening adverse effect of fentanyl is respiratory depression resulting from activation of μ-opioid receptors in the respiratory centers of the brain [5]. A number of experimental studies have shown that fentanyl can significantly reduce oxygen levels in the brain due to respiratory depression [6, 7]. These changes can lead to hypoxia, coma, acute toxic pulmonary edema, and death in severe cases of overdose. The fatal outcome in these cases is a consequence of direct toxic depression of the respiratory center in the brain, leading to apnea.

Development of the opioid crisis

The opioid epidemic has developed gradually over several decades and is often described in three waves [3, 8]. The first wave began in the 1990s and was associated with increased prescribing of opioids for chronic pain. During this period, the availability and use of prescription opioids increased significantly, leading to very high rates of opioid dependence. The second wave began around 2010 and opioid use was replaced by heroin use. The third wave began around 2013 and was associated with the illicitly produced synthetic opioids, fentanyl and its analogues [3, 8]. The final phase of the epidemic resulted in the largest increase in overdose deaths.

Statistical trends in opioid mortality

Between 1976 and 2016 in the United States, 599,255 were registered death cases from drug overdose [3]. In 2015, deaths opioid-related cases are were about 33,091 [9]. In 2023, about 105,000 people are deceased from overdose, nearly 80,000 of the cases are were caused from opioids. [8]

Increase in fentanyl-related deaths

Synthetic opioids, and fentanyl in particular, have become the leading contributors to fatal opioid overdoses. Fentanyl-related overdose deaths increased by 55% between 2015 and 2017 in New York City. The number of fatal overdoses, primarily from fentanyl and its analogues, was 19,547 in 2016 in the United States[3].Recent analyses have shown that fentanyl is the leading contributor to overdose deaths, surpassing both heroin and prescription opioids. Additional epidemiological reports indicate that opioid overdose deaths exceeded 74,000 in the 12-month period ending in April 2021, entering the third decade of the opioid epidemic [5].

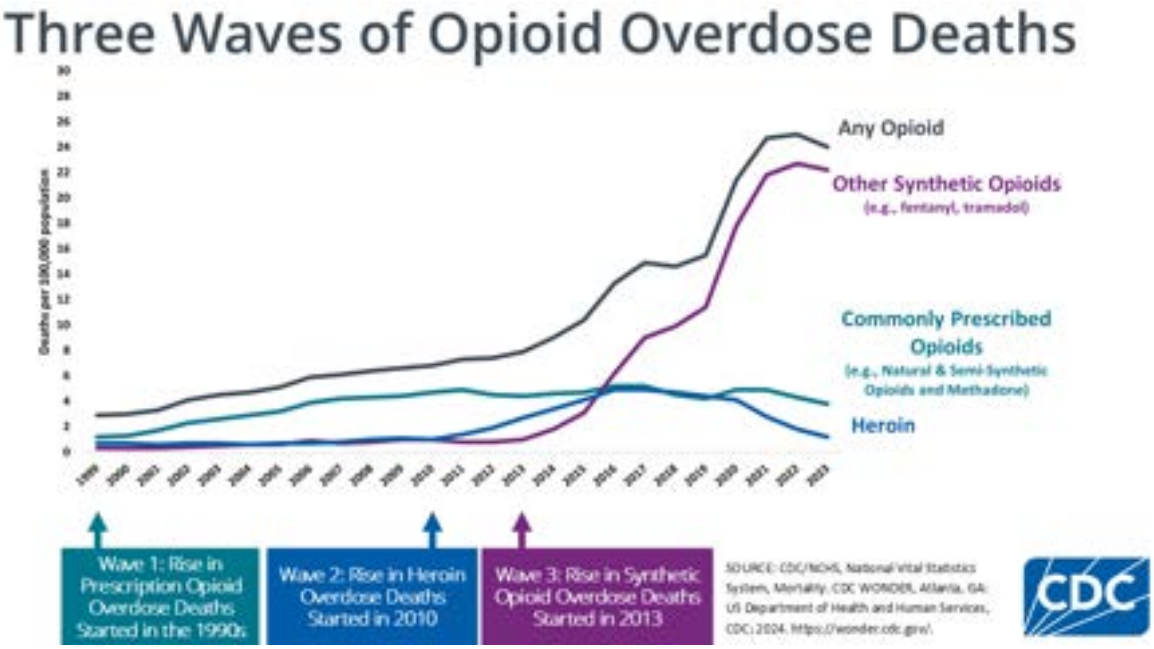

Figure 1. Trends in opioid overdose deaths in the United States (1999–2023). Reproduced from CDC [8]

Unintentional exposure to fentanyl

Important contributing factor for the growing mortals cases from overdose, is the widespread contamination of illegal fentanyl drugs without knowledge of the people [10]. The presence on Fentanyl in heroin, cocaine and counterfeit prescription pills, significantly increases the risk from heavy overdose.

Treatment

There is an argument that the treatment on fentanyl overdose treatment goes far beyond naloxone. Naloxone still remains the most important antidote, but highly potent synthetic opioids have led to additional therapeutic approaches [5]. Various pharmacological treatments currently being studied in humans include nalmefene, respiratory stimulants, and buprenorphine. Buprenorphine and methadone are also increasingly used after overdose to stabilize patients with opioid use disorder [3.11]. The latter reviews also discuss the potential treatment with higher doses buprenorphine, targeted especially to fentanyl and its analogues [12]. Current WHO guidelines emphasize the importance of supportive treatment, including airway management, oxygen therapy, and assisted ventilation in severe cases of overdose [13]. Adherence to these guidelines will lead to a reduction in opioid overdose deaths by improving patient care [13].

Discussion

The dual role of fentanyl illustrates the complex relationship between pharmaceutical drugs and dangerous public health consequences. Fentanyl remains a key drug for anesthesia and pain management, but its high potency and rapid onset make it particularly dangerous when used outside of medical settings. Illicit production of fentanyl has expanded because the drug is cheaply synthesized and transported in very small quantities due to its exceptional potency. The variability of illicit fentanyl production and the lack of dosage control significantly increase the risk of fatal overdose. Naloxone remains the primary pharmacological treatment for opioid overdose with autoinjectors and intranasal sprays [14]. As an opioid receptor

antagonist, naloxone reverses respiratory depression and can rapidly restore breathing [15]. However, due to the high potency of fentanyl, multiple doses of naloxone may sometimes be required [15]. Public health responses to the fentanyl crisis include improved drug monitoring, harm reduction programs, wider availability of naloxone, and public education on overdose prevention.

Conclusion

Fentanyl is a prime example of the "dual" nature of modern pharmacology. While it provides powerful pain relief, its high potency and growing presence in illicit drug markets make it a dangerous contributor to the opioid crisis. Statistics show a significant increase in fentanyl-related overdose deaths over the past decade. Addressing this crisis requires a balanced strategy that supports the legitimate medical use of fentanyl while implementing effective measures to prevent abuse and overdose deaths.

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Differentiated thyroid cancer and the importance of early diagnosis – a case report

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Introduction: Papillary thyroid cancer originates from thyroid follicular cells and is the most common type of thyroid carcinoma. Although it has a favourable prognosis, early diagnosis and treatment are essential for working towards the best possible outcome for the patient. **Case description:** We present a case of a 27-year-old woman who was referred for a check-up regarding polycystic ovaries. Thyroid ultrasound revealed a hypoechogenic formation with an irregular shape, blurred margins and microcalcifications with sizes 10.3/5.5/9.4 mm in the isthmic part of the right lobe, suspicious for capsular invasion. 2 oval lymph nodes were also detected posterior to the right lobe at level VI with hypoechogenic structure and increased intranodular blood flow with a maximum diameter of 4 mm. Fine-needle aspiration biopsy (FNAB) of the high-risk formation was performed, which confirmed Bethesda V. Results from the biopsy of the lymph nodes in level VI were suspicious for metastasis. Wash-out analysis for thyroglobulin (> 1000 ng/ml) and calcitonin (3.403 pg/ml) was performed, confirming metastasis from differentiated thyroid cancer. The patient underwent thyroidectomy with central lymph node dissection. Histology identified metastatic papillary thyroid cancer with a diameter of 7 mm, without capsular invasion. **Conclusion:** Papillary thyroid carcinoma has slow growth and progression in most cases. However, in some cases, despite being small in size, regional lymph node metastasis can occur. Therefore, the precise preoperative diagnosis of lymph node metastasis by US, cytology and wash-out analysis is crucial. Early surgical treatment and post-operative management work together to prevent complications.

Keywords: *thyroid cancer, metastases, FNA, wash-out analysis*

Introduction and aim

Differentiated thyroid cancer is the most common type of thyroid cancer, originating from the thyrocytes. Overall, it has a favourable prognosis due to its slow growth and progression. The aim of this case report is to depict the occurrence of metastasis in even small-sized malignancies and highlight the importance of early diagnosis in differentiated thyroid cancer.

Case Presentation

A 27-year-old female patient was referred for a routine check-up for polycystic ovaries. Her main complaints were irregular menstrual cycle, whereas regarding her thyroid gland, no symptoms were present. She was clinically and hormonally euthyroid (Table 1).

Table 1. Thyroid function tests

Parameter	Result	Reference ranges
TSH (mU/L)	2.033	0.38–5.33
fT4 (pmol/L)	9.66	7.86–14.41
Anti TPO (U/mL)	1.2	0–20

An ultrasound of her thyroid gland was made, which revealed suspicious findings. A high-risk hypoechogenic formation with irregular shape, blurred margins and microcalcification (category EU-TIRADS 5), measuring 10.3/5.5/9.4 mm within the isthmic part of the right lobe was observed (Fig. 1). There was also suspicion for capsular invasion. Additionally, posterior to the right lobe, two round hyperechoic lymph nodes with lack of hilum were detected (Fig. 1). Furthermore, fine needle aspiration biopsy (FNAB) was performed. The cytological analysis of the thyroid lesion indicated Bethesda V category and confirmed malignancy of one of the lymph nodes. Washout analysis of the lymph node presented markedly elevated thyroglobulin levels > 1000 ng/mL and negative calcitonin – 3.403 pg/mL, which suggested differentiated thyroid cancer. The patient underwent total thyroidectomy with central lymph node dissection, after which histological analysis confirmed metastatic papillary thyroid carcinoma, which was 7 mm in diameter.

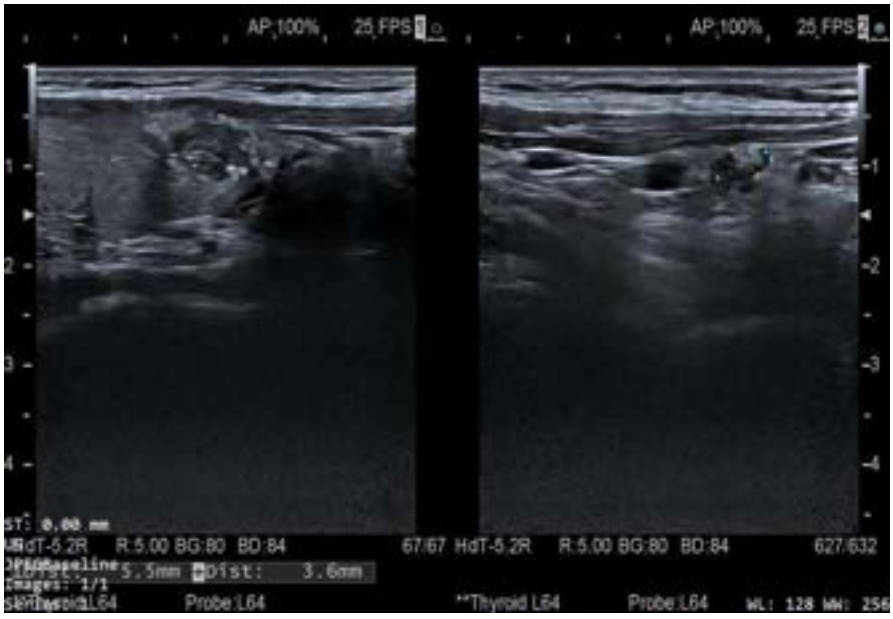

Figure 1. Ultrasound of the neck area: the high-risk thyroid nodule (EU-TIRADS 5) on the left part of the picture, and a suspicious hyperechoic lymph node on the right part of the picture.

Discussion

The incidence patterns of thyroid carcinoma, especially microcarcinoma, has risen owing to improved diagnostic clarity [1]. This trend is reflected in this case as the thyroid nodule was discovered incidentally during a check-up due to polycystic ovarian syndrome. A constellation of characteristics associated with high risk of malignancy was found in thyroid ultrasound – hypoechogenic nodules, irregular margins, blurred capsular outline and microcalcifications. According to ETA and ATA, the presence of these features suggests that an FNAB should be performed [2, 3]. The nodule was 10.3/5.5/9.4 mm in size, located in the isthmic part of the right lobe. FNAB corresponded to cytological category Bethesda V, thus surgical intervention was required. Accordingly, thyroidectomy with central lymph node dissection was performed from which subsequent histological findings, in hand with the cytological results helped confirm the diagnosis of papillary thyroid carcinoma. Therefore, this case illustrates the ability of thyroid malignancies as small as 7 mm to metastasise.

Tumour size is not the only and pivotal predictor for metastatic disease, as tumour biology plays a key role [4]. Two oval hyperechoic lymph nodes with lack of hilum were detected. FNAB cytology of one of the lymph nodes was performed, followed by thyroglobulin washout analysis. The result was over 1000 ng/ml, which determined metastatic lymph node involvement. Different thresholds for thyroglobulin wash-out have been proposed, such as absolute values as low as 1.8 ng/ml [5] or thyroglobulin wash-out/serum thyroglobulin ratio [6]. Although currently there are no internationally recognized cut-offs for wash-out analysis for thyroglobulin, the method is extensively used due to its high diagnostic accuracy. It allows an adequate and timely diagnosis of metastases, preoperative confirmation of the histological type of cancer and fosters optimal planning of the scope of the surgery.

Conclusion

This case of an incidentally discovered papillary thyroid carcinoma with metastasis highlights the necessity of a thorough

diagnostic approach to thyroid nodules, regardless of the manner of their discovery. Accurate preoperative staging can be achieved by ultrasound, cytology and washout-analysis. Timely intervention and appropriate treatment can be provided, allowing for optimal patient outcome.

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A rare case of prostate cancer initially presenting with rectorrhagia

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Rectorrhagia, defined as the passage of fresh blood per rectum, is a frequent clinical complaint most often attributed to benign anorectal conditions or primary colorectal pathology. Malignant causes are less common and rarely originate outside the gastrointestinal tract. Prostate cancer is the second most prevalent malignancy among men worldwide and typically presents with urinary symptoms or is detected through prostate-specific antigen testing. Presentation with rectal bleeding as an initial symptom is exceptional. We report a rare case of prostate adenocarcinoma initially manifesting as persistent rectorrhagia. A male patient in his seventh decade presented with a two-month history of rectal bleeding. Surgical evaluation ruled out hemorrhoidal disease. Subsequent colonoscopy demonstrated infiltrative changes of the rectal mucosa, raising suspicion of a primary rectal neoplasm. Histopathological examination of rectal biopsy specimens, however, revealed adenocarcinoma of prostatic origin. Further assessment with multiparametric magnetic resonance imaging identified a large locally advanced prostate tumour with direct invasion of the rectum and the urinary bladder. This case illustrates that prostate cancer may present with symptoms arising from adjacent pelvic organs rather than the genitourinary system. In male patients with unexplained rectorrhagia, particularly when standard causes are excluded, malignancies of the small pelvis should be considered in the differential diagnosis.

Keywords: *rectorrhagia, prostate, cancer*

Introduction

Rectorrhagia, defined as the passage of fresh blood per rectum, is a common clinical presentation that may signal a range of underlying pathologies, ranging from benign anorectal conditions to colorectal malignancy (1). Extraintestinal sources are rare and may present a diagnostic challenge.

Among these, prostate cancer presenting with rectal bleeding is exceptionally rare and has been described only in a limited number of cases (3, 4, 5). Despite the close anatomical relationship between the prostate and rectum, direct rectal involvement is uncommon and generally occurs only in advanced disease, where it may mimic primary colorectal pathology (3, 5). In such atypical presentations, imaging plays a pivotal role in distinguishing primary gastrointestinal pathology from secondary involvement by adjacent pelvic malignancies.

Case presentation

We report the case of a male patient presenting with persistent rectorrhagia over a two-month period, as his initial clinical complaint. The patient denied any lower urinary tract symptoms, including urinary urgency, dysuria, or hematuria.

On admission, the patient was hemodynamically stable and afebrile and physical examination was unremarkable. Initial surgical evaluation did not demonstrate evidence of hemorrhoidal disease.

In light of the persistence of symptoms, further gastroenterological assessment was undertaken. Colonoscopic examination revealed infiltrative changes involving the rectal mucosa, raising suspicion of a primary rectal malignancy. Histopathological analysis, however, demonstrated adenocarcinoma of prostatic origin, with a Gleason score of 3 + 4 = 7.

Laboratory evaluation revealed a markedly elevated prostate-specific antigen (PSA) level of 96.47 ng/mL, as well as anemia and elevated lactate dehydrogenase levels, consistent with advanced disease. Further evaluation with imaging was subsequently performed to assess the extent of disease.

Diagnostic imaging

Multiparametric magnetic resonance imaging (MRI) of the pelvis demonstrated a lobulated, infiltrative lesion centered in the prostate gland, measuring approximately 32 × 36 mm. The lesion exhibited low signal intensity on T2-weighted sequences, marked diffusion restriction on DWI/ADC sequences, and post-contrast enhancement, consistent with malignancy.

There was direct invasion into the rectal wall, urinary bladder, seminal vesicles, and mesorectal fascia, explaining the patient's presenting symptom of rectorrhagia. Regional disease involvement was evident, with enlargement of perirectal lymph nodes, and right inguinal lymphadenopathy.

Multiparametric MRI was essential in identifying the tumour origin, assessing local invasion, and differentiating secondary rectal involvement from primary rectal pathology (6) (Figure 1).

Subsequent cross-sectional imaging revealed widespread metastatic dissemination, including multiple hepatic metastases, extensive lymphadenopathy, osseous metastases involving the vertebral bodies and ribs, and additional metastases involving the pancreas, kidneys, and psoas muscle (Figure 2).

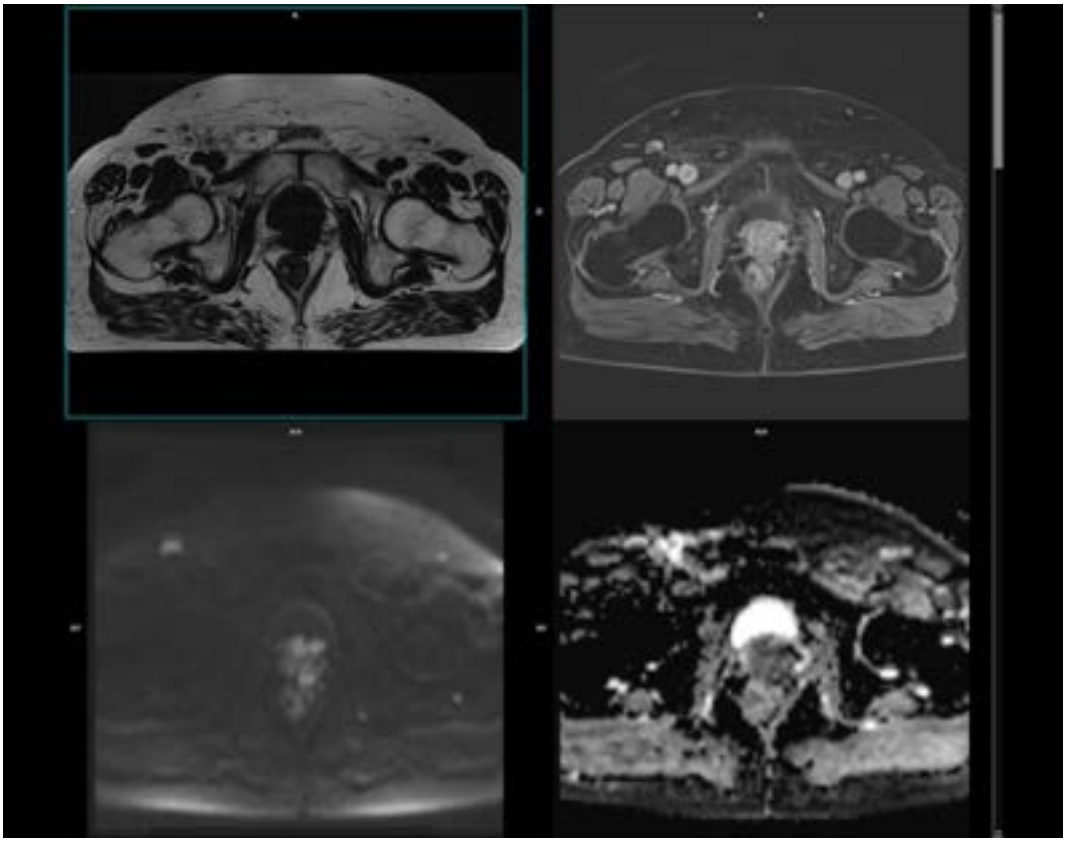

Figure 1. T1; T1 + contrast; DWI; ADC

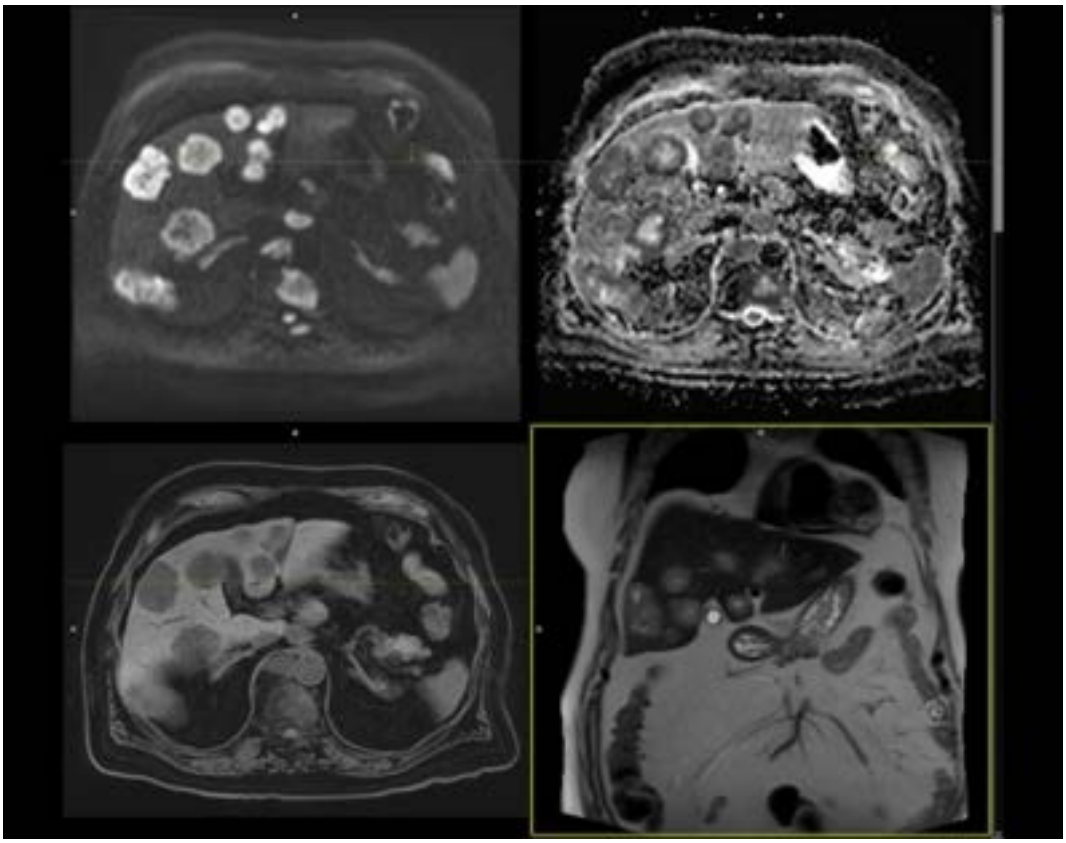

Figure 2. DWI; ADC; T1; T2 – liver metastases, vertebral metastases, lymph node metastases

Overall, imaging findings were consistent with advanced prostate carcinoma with local invasion, nodal involvement, and distant metastatic disease (cT3 cN1 cM1).

The patient was managed with androgen deprivation therapy and second-line hormonal agents, with palliative radiotherapy administered for symptomatic osseous metastases.

Discussion

Although rectorrhagia is most commonly associated with gastrointestinal pathology, its occurrence as the initial manifestation of prostate cancer is exceptionally rare and may mimic primary colorectal malignancy (3, 4, 5). Reported cases typically occur in the setting of locally advanced disease with direct rectal invasion.

Prostate cancer is the second most commonly diagnosed malignancy in men worldwide (2), yet many patients remain asymptomatic even in advanced stages of disease. In this case, the absence of lower urinary tract symptoms delayed suspicion of a prostatic origin. Rather than reflecting primary gastrointestinal pathology, rectorrhagia resulted from direct tumour invasion into adjacent pelvic structures.

This pattern of spread can be explained by tumour origin in the peripheral zone of the prostate, allowing posterior extension through Denonvilliers' fascia into the rectal wall without early urethral obstruction (3, 5).

Imaging played a central role in establishing both the origin and extent of disease. While colonoscopic findings initially suggested primary rectal malignancy (4, 7), multiparametric MRI enabled accurate identification of the prostatic tumour and delineation of local invasion into surrounding pelvic structures (6). Cross-sectional imaging

additionally demonstrated nodal and distant metastatic disease, confirming advanced-stage carcinoma.

Such presentations are often associated with advanced disease and poorer prognosis (3, 5). This case highlights the importance of considering extraintestinal malignancies in male patients presenting with unexplained rectorrhagia, particularly when clinical and endoscopic findings are discordant.

Conclusion

Prostate cancer may rarely present with symptoms arising from local invasion, such as rectorrhagia, rather than the genitourinary system. This atypical presentation may mimic primary colorectal pathology and delay recognition of a prostatic origin. In such cases, multiparametric MRI plays a central role in identifying tumour origin, assessing local invasion, and staging disease. Awareness of these uncommon presentations is essential to facilitate appropriate management.

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In search of a parathyroid adenoma – a case report

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Introduction: Primary hyperparathyroidism (PHPT) is characterised by elevated or inappropriately normal serum parathyroid hormone, leading to hypercalcemia and multisystem involvement. **Case presentation:** A 64-year-old female was referred to the Endocrinology Clinic for assessment due to hypercalcemia and recurrent nephrolithiasis. Scintigraphy with 99mTc-sestamibi had been performed, with a negative result. Laboratory studies revealed a constellation consistent with PHPT. Ultrasound of the neck revealed a thyroid gland of normal size, shape, echogenicity, and vascularisation. Several hypoechoic formations were observed posterior to the thyroid lobe and one high-risk nodule in the lower part of the left lobe. Fine-needle aspiration (FNA) with parathyroid hormone washout analysis was performed. PTH-washout analysis was negative for the extrathyroid formations; however, it was markedly elevated in the sample from the intrathyroid nodule. The cytological results of all formations were Bethesda II category. The patient underwent successful partial thyroid resection with removal of the parathyroid adenoma and normalisation of serum calcium levels. **Conclusion:** PTH measurement in fine-needle aspiration washout fluid is a highly sensitive and specific method for the diagnosis of parathyroid adenomas. It is exceptionally valuable in cases of small lesions, which can be missed on scintigraphy. The possibility of simultaneous cytological and functional assessment of the formation is of great interest, especially in cases of intrathyroid location of parathyroid adenomas.

Keywords: *parathyroid adenoma, fine-needle aspiration biopsy, wash-out analysis*

Introduction

Primary hyperparathyroidism (PHPT) is an endocrine disorder characterized by elevated parathyroid hormone (PTH) levels leading to hypercalcemia and multisystem involvement [1]. In majority of the cases, a parathyroid adenoma is the leading cause (85%) [1], followed by multiglandular disease (15%), or parathyroid carcinoma (1%). Historically, classic presentation of PHPT consisted of signs – stones, bones, abdominal groans and psychiatric overtones – referring to nephrolithiasis, osteoporosis/osteitis fibrosa cystica, peptic ulcer disease, and/or severe neuropsychiatric symptoms – indicating patient diagnosed at a later stage of the disease [1]. In previous years, the common clinical presentation involved symptomatic renal or skeletal disease with moderate or severe hypercalcemia [1]. However, in more recent cases patients present with fewer or no symptoms and the diagnosis is established incidentally [1]. Standard radiological assessment includes scintigraphy of parathyroid and thyroid glands with 99mTc-sestamibi and ultrasound (US) of the neck [1]. However, these standard diagnostic modalities can test negative making localisation of the lesions difficult, therefore other diagnostic methods are considered such as PTH washout analysis [4, 5]. The following study presents a case report of PHPT with an intra-thyroid parathyroid adenoma. It highlights diagnostic challenges when standard imaging probes are negative and put emphasis on the PTH washout analysis as a technique for localisation of parathyroid tissue [1].

Case presentation

A 64-year-old female, with no associated family history, was referred to an Endocrinology Clinic for assessment due to hypercalcemia and recurrent nephrolithiasis. She was referred for a scintigraphy with 99mTc-sestamibi, however no hyperactive parathyroid glands were noted [1]. Nevertheless, her laboratory tests revealed a constellation for PHPT: elevated serum PTH – 126 pg/ml (12–88) and serum Ca – 2.68 mmol/L (2.17–

2.62), low serum phosphate levels – 0.84 mmol/L (0.72–1.35) [1]. Creatinine levels were normal – 98 μmol/L (42–110) and mild vitamin D hypovitaminosis was observed – 25.97 ng/mL (30–100) [1]. The ultrasound of the neck area revealed a thyroid gland of normal size, shape, echogenicity, and vascularization [3]. Several subcentimeter hypoechoic formations with increased blood flow were observed: two posterior to the thyroid gland bilaterally (Fig. 1) and one markedly hypoechoic high-risk nodule in the lower part of the left lobe – category EU-TIRADS 5 (Fig. 2) [3]

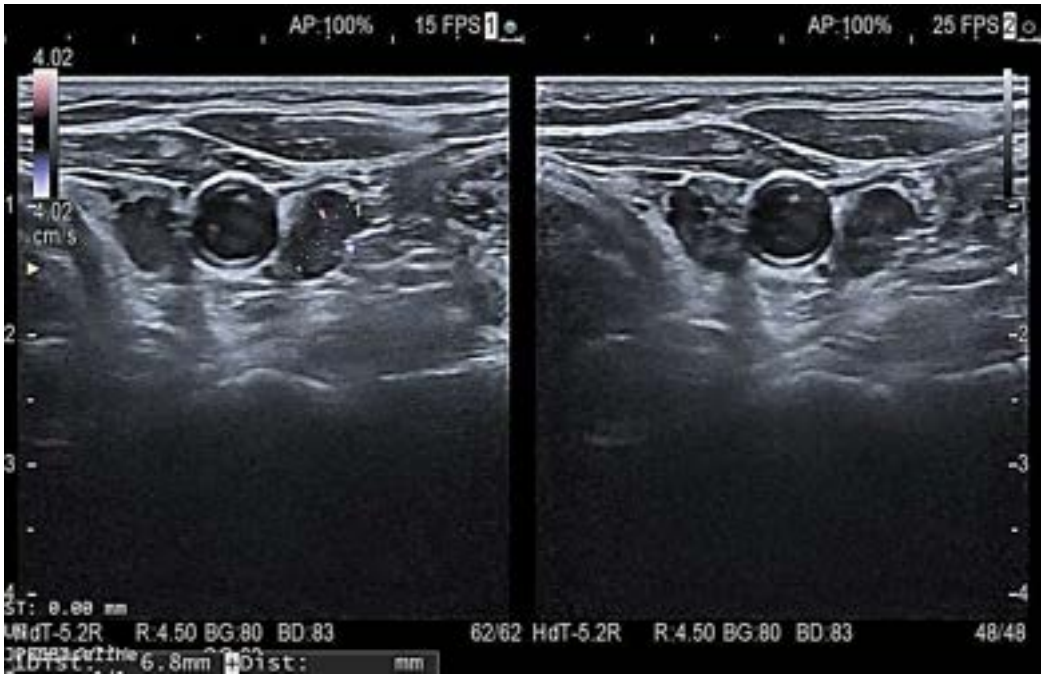

Figure 1. Ultrasound presentation of the hypoechoic formation, posterior to the left lobe, d = 6.8 mm, with a negative PTH-washout

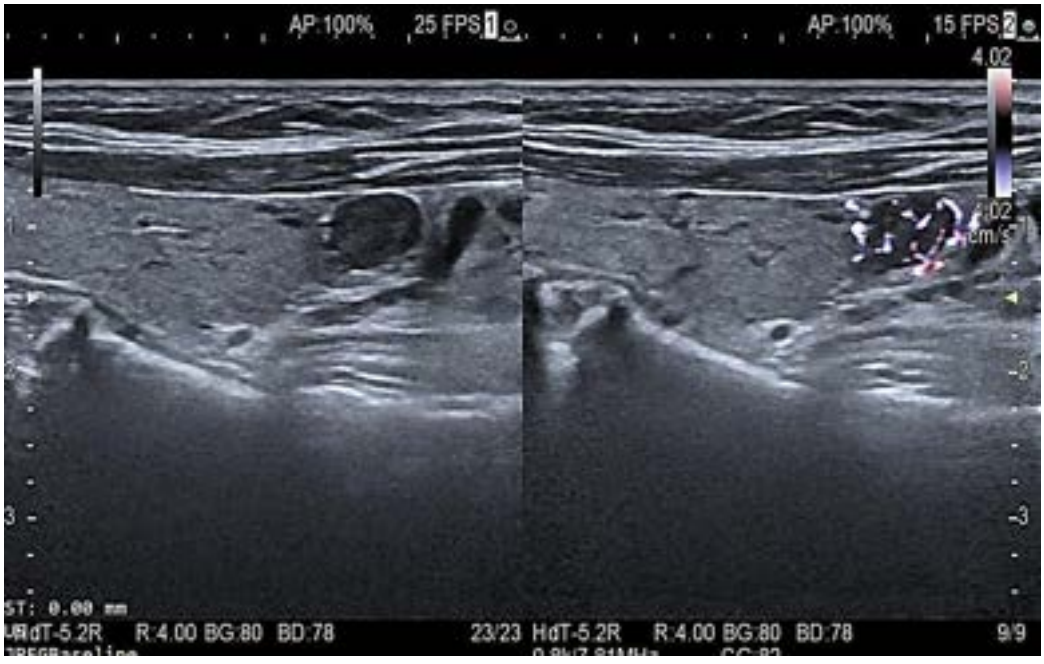

Figure 2. Ultrasound presentation of the markedly hypoechoic high-risk nodule in the lower third of the left lobe, d = 7 mm, category EU-TIRADS 5 [3], with a positive PTH-washout

To determine the nature of the lesions, a fine-needle aspiration biopsy (FNA) with PTH washout analysis was performed [4, 5]. All biopsied formations were benign, Bethesda II category. The extrathyroid lesions located posterior to the right lobe and posterior to the left lobe showed negative PTH-washout – 3.4 pg/mL and 10.8 pg/mL, respectively. In contrast, the intrathyroid lesion in the lower part of the left thyroid lobe had a significantly high PTH level of 2735.5 pg/mL, proving a parathyroid adenoma [4, 5].

Consecutively, the patient was referred for a partial thyroid resection to remove the intrathyroid parathyroid adenoma. The surgery was successful, with no postoperative complications, except for mild transitory hypoparathyroidism [1, 4], which is often observed after removal of a parathyroid adenoma. This can be due to the fact that the hyperfunctioning parathyroid tissue has led to decreased function of the rest of the parathyroid glands. After removal, there is a recovery period during which the healthy glands need time to regain function, leading to a temporary drop in PTH levels. Postoperative PTH decreased to 11.1 pg/mL (12 to 88 pg/mL), indicating the success of the surgery. Calcium and active vitamin D supplements were provided to prevent hypocalcaemia and hungry bone syndrome [1].

PTH washout is a sensitive and specific method for identifying parathyroid tissue. It is especially useful when standard imaging like SPECT-CT or scintigraphy is negative or inconclusive [4, 5]. Like any diagnostic method, it has its pros and cons. The advantages include very high specificity for parathyroid tissue. It detects the PTH hormone directly in the aspirated fluid, which helps differentiate parathyroid lesions from thyroid nodules. The method is cost-effective and provides a timely diagnosis simultaneously with cytological assessment of the formation. It is minimally invasive, precise and done under ultrasound guidance, reducing the need for localizing surgery. It helps locate ectopic and intrathyroidal glands, which is important in challenging cases. However, it has limitations [4, 5]. There is no universally accepted cutoff value, which can lead to confusion, as interpretation varies between labs. Some consider mildly elevated PTH in aspirate as positive, while others may only count extremely high levels as positive, causing inconsistent interpretation and potential misdiagnosis across different hospitals and labs. This is a FNA technique, so it requires skilled personnel and careful lab handling. Contamination with blood or surrounding tissue must be avoided, as it can affect accuracy. The use of a fine needle carries a minimal risk of bleeding or hematoma from puncturing vascular neck structures. There is also a low risk of false negatives, which may occur due to sampling errors. A very rare risk associated with the PTH washout method is parathyromatosis, a condition where multiple small foci of functioning parathyroid tissue are scattered in the neck or surrounding tissues following the trace of the needle, leading to persistent recurrent hyperparathyroidism [4, 5].

In summary, PTH washout is a highly sensitive and specific method for confirming parathyroid tissue [4, 5]. It is a valuable diagnostic tool for small lesions, particularly those located intrathyroidal, which might be missed by scintigraphy [4, 5]. It assists in accurately localizing difficult cases, such as intrathyroidal parathyroid adenomas when standard imaging is inconclusive [4, 5].

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Eosinophilic granulomatosis with polyangiitis – present treatment guidelines

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Introduction: Eosinophilic granulomatosis with polyangiitis previously known as a Churg-Strauss syndrome is a rare multisystem inflammatory disease. The main characteristics of the disease are late onset asthma, tissue and blood eosinophilia and necrotizing inflammation of small and medium sized vessels. Delayed diagnosis may lead to irreversible organ damage, mostly of the heart, lungs, peripheral nerves and kidneys. Recent advances in immunology have thrown light on the central role of type 2 helper T cell driven inflammation and eosinophil activation. **Aim:** To summarize and evaluate the new therapeutic approaches and targeted treatments for eosinophilic granulomatosis with polyangiitis. **Materials and methods:** A literature-based review on latest clinical trials, observational studies and recent recommendations focusing on induction the remission, maintenance therapy and the prevention of relapses. **Results and discussion:** Systemic glucocorticoids remain the main therapy for initial treatment and control of the inflammation. Patients with poor prognostic factors require additional immunosuppressive therapy, typically with cyclophosphamide for remission induction followed by azathioprine or methotrexate for the following maintenance. The frequent relapses and long term glucocorticoid toxicity present major therapeutic challenges. Targeted biological agents directed against interleukin five or its receptor significantly reduce eosinophil counts and prolong remission. They are decreasing relapse rates and lead to glucocorticoid reduction. The new therapeutic agents also improve asthma control and provide better quality of life. **Conclusion:** Modern approaches for eosinophilic granulomatosis with polyangiitis are combination of the standardized immunosuppression therapy and targeted biological therapy, improving the quality of life of the patients and reducing the side effects of steroid toxicty.

Keywords: *eosinophilic vasculitis, severe asthma, biological therapy*

Introduction

Eosinophilic granulomatosis with polyangiitis (EGPA), formerly known as Churg-Strauss syndrome, is a rare antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis that primarily affects small and medium-sized vessels [1]. It is characterized by tissue eosinophilia, eosinophilic granulomatous inflammation, and necrotizing vasculitis involving multiple organs. Clinically, it is associated with a "classic triad" of late-onset asthma and/or allergic rhinitis, eosinophilic lung disease, and systemic vasculitis, which can lead to manifestations such as mononeuritis multiplex, peripheral neuropathy, and peripheral eosinophilia [2, 3]. The disease most commonly presents between the second and fourth decades of life [4]. EGPA classically progresses through three clinical phases: a prodromal allergic phase, characterized primarily by asthma and allergic rhinitis [5], followed by an eosinophilic phase, and subsequently a vasculitic phase, involving systemic inflammation of small and medium-sized vessels [6]. These phases often overlap and may not occur sequentially, with many patients presenting with multiorgan manifestations that complicate early diagnosis [7]. Rapid recognition and early initiation of treatment are essential, as delay in diagnosis can lead to irreversible cardiac, pulmonary, neurological, and vascular damage. Conventional treatment of EGPA relies primarily on glucocorticoids and immunosuppressive agents. Long-term therapy, however, is often associated with relapse, cumulative toxicity, and glucocorticoid dependence. Treatment strategies are increasingly shifting toward targeted biological therapies directed against

interleukin-5 (IL-5), a key cytokine involved in the activation, maturation, and survival of eosinophils [5].

Aim

To systematize the clinical and pathological characteristics of eosinophilic granulomatosis with polyangiitis (EGPA) by evaluating current therapeutic approaches, focusing on the transition from conventional immunosuppressive treatment to targeted biological therapy with anti-IL-5/IL-5 receptor.

Materials and methods

The literature review was conducted using peer-reviewed articles, with a preference for publications within the last five years. Sources were identified through PubMed, Google Scholar, and relevant journal databases using search terms including eosinophilic granulomatosis with polyangiitis, EGPA, interleukin-5, anti-IL-5 therapy, glucocorticoids, mepolizumab, benralizumab, and Churg-Strauss syndrome.

Results

Clinical and pathological characteristics

EGPA begins with a prodromal stage characterized by atopic or allergic manifestations, most notably asthma and chronic rhinosinusitis [4]. This is followed by an eosinophilic stage that may persist for several years before vasculitic manifestations develop. During this phase, eosinophilic tissue infiltration occurs, affecting the lungs, heart, and gastrointestinal tract. Eosinophilic cardiomyopathy, pulmonary infiltrates, and gastrointestinal disorders develop [9]. The vasculitic stage is characterized by eosinophilic granulomatous inflammation and necrotizing vasculitis affecting small and medium-sized vessels, with predominant involvement of the skin, nervous system, and kidneys. The clinical manifestations are vascular purpura and mononeuritis multiplex, presenting with foot drop and symmetrical polyneuropathy [9]. Eosinophilic myocarditis and cardiomyopathy are major causes of mortality associated with EGPA [10].

Treatment challenges in conventional therapy

Glucocorticoids, such as prednisone and methylprednisolone, are the first-line treatment in the traditional therapy of EGPA. Prolonged exposure to glucocorticoids is associated with significant cumulative toxicity, including glucocorticoid-induced osteoporosis and risk of fractures [11]. Long - term glucocorticoid therapy can lead to steroid dependence, with studies reporting glucocorticoid dependence in approximately 79–85% of patients with EGPA. In addition, conventional glucocorticoid-based therapy does not completely prevent disease relapse, with approximately 25–42% of patients experiencing relapse or requiring additional immunosuppressive therapy [12]. Immunosuppressive agents such as cyclophosphamide may be used in patients with poor prognosis or organ-threatening EGPA, especially when glucocorticoid therapy alone is insufficient to control active vasculitic disease and achieve remission [8]. Cyclophosphamide acts as an alkylating agent that suppresses rapidly proliferating immune cells, thereby inhibiting immune cell replication and reducing the autoimmune and vasculitic processes underlying EGPA [13]. Cyclophosphamide can improve disease control and remission rates, but long-term use is limited by adverse effects, including increased risk of infections, malignancies, and infertility [13]. Azathioprine is commonly used during the maintenance phase of EGPA treatment to reduce relapse rates, lowering glucocorticoid doses, and minimize prolonged exposure to cyclophosphamide. It

functions through purine metabolic pathways to reduce DNA synthesis in proliferating immune cells, thereby limiting persistent immune activation [14]. Although considered safer than cyclophosphamide for long-term therapy, azathioprine is associated with significant toxicity, particularly bone marrow suppression and hepatotoxicity [14].

Biological therapy targeting IL-5

Given the limited long-term efficacy of conventional treatments for EGPA, treatment has increasingly shifted towards targeting the interleukin-5 (IL-5) pathway. IL-5 is a key inflammatory cytokine produced primarily by T helper 2 (Th2) cells that plays a fundamental role in the activation, maturation, and mobilization of eosinophils from the bone marrow [5]. Current therapies targeting IL-5 or its corresponding receptor can reduce eosinophilic tissue infiltration and subsequent organ damage. Mepolizumab, a humanized monoclonal antibody against IL-5, acts by directly binding to free IL-5 in the circulation, thereby preventing its binding to the interleukin-5 receptor alpha (IL-5Rα) expressed on eosinophils. This reduces the activation and survival of eosinophils in the body, thereby reducing tissue eosinophilia and organ infiltration [15]. A 52-week phase III randomized controlled trial (RCT), known as the MIRRA study, investigated 136 patients with relapsed or refractory EGPA who were assigned to receive either mepolizumab 300 mg every 4 weeks in addition to standard therapy or placebo with standard therapy. Patients treated with mepolizumab demonstrated significantly improved outcomes, with 28% achieving at least 24 weeks of sustained remission compared with only 3% of individuals in the placebo group. The study also demonstrated a significant benefit from glucocorticoid reduction, as 44% of patients receiving mepolizumab were able to reduce their prednisolone or prednisone dose to 4 mg/day or less at weeks 48–52, compared with 7% of patients receiving placebo [16]. Benralizumab (Fasenra), a humanized monoclonal IgG antibody directed against interleukin-5 receptor alpha (IL-5Rα), is another targeted biologic therapy used in the treatment of EGPA. The drug exerts its therapeutic effect by directly binding to IL-5Rα expressed on eosinophils, thereby inhibiting IL-5-mediated eosinophil activation and signaling [17]. In addition, benralizumab contains an Fc region that interacts with the FcγRIIIa receptor present on natural killer (NK) cells, triggering antibody-dependent cellular cytotoxicity (ADCC) and subsequent eosinophil apoptosis. This results in a significant reduction in tissue eosinophilia and organ infiltration [17]. In the phase III SIROCCO trial, benralizumab was shown to reduce annual asthma exacerbation rates by up to 51% over 48 weeks while improving pre-bronchodilator FEV1 by up to 159 mL in patients with severe eosinophilic asthma [18].

Discussion

The systematized data in the review focuses mainly on the main therapeutic challenges associated with EGPA, and in particular emphasize the cumulative toxicity and long-term limitations of conventional therapy. Although glucocorticoids remain highly effective in inducing initial remission of the disease, they do not adequately prevent its relapse for prolonged periods. As a result, many patients require prolonged use of steroids, which increases the risk of glucocorticoid dependence and associated adverse drug reactions. Similarly, immunosuppressive drugs such as cyclophosphamide and azathioprine can improve disease remission and reduce the need for glucocorticoid therapy, especially in severe forms of EGPA. Modern treatment of EGPA is shifting towards biological therapies that

specifically target the central driver of eosinophilic inflammation: the IL-5 pathway. Promising results from clinical trials, such as the MIRRA and MANDARA studies, have shown that mepolizumab and benralizumab significantly improve remission rates of EGPA while reducing glucocorticoid requirements.

Conclusion

Despite these promising advances, biological therapies remain relatively new and further long-term studies are needed to assess their safety, affordability, durability, and cost-effectiveness. Therefore, a combination therapeutic approach involving conventional immunosuppressive therapy in combination with targeted biological agents may provide better long-term disease control in patients with EGPA.

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Extraluminal gallstones on computed tomography as a diagnostic clue to gallbladder perforation: a case report

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Spontaneous gallbladder perforation is a rare but life-threatening condition that often presents with non-specific clinical features of acute diffuse peritonitis, making early diagnosis challenging. Imaging plays a critical role in timely recognition, with computed tomography (CT) being the most sensitive modality for detecting gallbladder wall disruption and associated complications. This report presents a case of a male patient with acute diffuse abdominal pain and signs of peritoneal irritation, with a background of liver cirrhosis, hepatitis C, and portal hypertension. Emergency non-contrast CT of the abdomen and pelvis revealed an ill-defined gallbladder with increased intraluminal attenuation, large-volume intraperitoneal free fluid, and, most notably, extraluminal gallstones located in the subhepatic region and within the pelvic ascites. Comparison with a CT examination performed several months earlier revealed the same gallstones previously located intraluminally within the gallbladder, confirming their migration into the peritoneal cavity. The identification of extraluminal gallstones, supported by comparison with prior imaging, was decisive for the diagnosis of gallbladder perforation. This case highlights the value of CT, including comparative imaging, in achieving early and confident diagnosis of this rare cause of acute peritonitis and guiding urgent clinical management.

Keywords: *gallbladder, perforation, computed tomography, extraluminal gallstones, peritonitis*

Introduction

Gallbladder perforation (GB) is a rare but serious complication, most commonly associated with acute cholecystitis (1, 2). Its clinical presentation is often nonspecific and can mimic other causes of acute abdomen, which makes early diagnosis challenging and may delay treatment (1, 3). Imaging therefore plays a central role, with CT considered the most sensitive modality for identifying gallbladder wall disruption and associated findings (2). This case report highlights the diagnostic value of CT in the timely diagnosis of gallbladder perforation and emphasizes the importance of comparing current and prior imaging to increase diagnostic confidence.

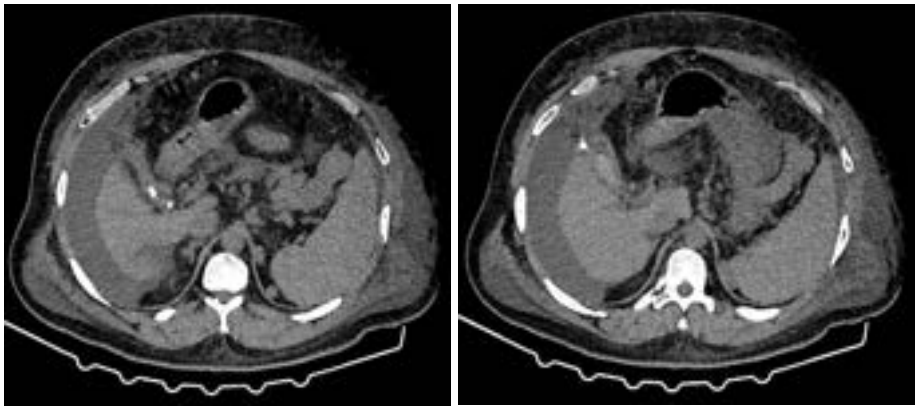
Case presentation

A male patient presented to the gastroenterology department with diffuse abdominal pain lasting several hours, accompanied by nausea. His medical history included liver cirrhosis, chronic hepatitis C, portal hypertension with grade I esophageal varices, chronic alcohol abuse, and active smoking.

On physical examination, he appeared in poor general condition, with pale, icteric skin and mucous membranes. The abdomen was distended, with diffuse tenderness, guarding and signs of peritoneal irritation. Bowel sounds were hypoactive on auscultation. Laboratory tests showed leukocytosis (WBC: 20×10^9/L), elevated C-reactive protein (CRP: 150 mg/L), hyperbilirubinemia (70 μmol/L), and impaired liver function tests.

An emergency non-contrast CT scan of the abdomen and pelvis demonstrated an ill-defined gallbladder with increased intraluminal attenuation, a large amount of intraperitoneal free fluid and multiple extraluminal gallstones in the subhepatic region and within pelvic ascites, measuring up to 1 cm (Fig. 1 A-B).

A.



B.

Figure 1. Non-contrast axial CT images demonstrating an ill-defined gallbladder with extraluminal gallstones in the subhepatic region (A) and hyperdense gallstones within pelvic ascitic fluid (B).

Comparison with a prior CT scan demonstrated the same gallstones previously located within the gallbladder, confirming their migration into the peritoneal cavity (Fig. 2). Taken together, these findings were highly suggestive of gallbladder perforation with diffuse peritonitis.

Figure 2. Prior non-contrast CT examination showing the same gallstones located intraluminally within the gallbladder.

Discussion

Gallbladder perforation is a rare but potentially life-threatening complication, most often associated with acute cholecystitis, with reported mortality rates ranging from 12% to 42% (1, 3). Early diagnosis remains challenging because the clinical presentation is often nonspecific and may overlap with other causes of acute abdomen (1, 3).

According to the Niemeier classification, gallbladder perforation is divided into acute, subacute, and chronic type (4). Based on the imaging and clinical findings, our case is consistent with the acute type, the most severe presentation.

Imaging plays a central role in diagnosis, particularly when clinical findings are inconclusive. CT is considered the most sensitive modality for detecting gallbladder perforation, as it can demonstrate both direct signs,

such as gallbladder wall discontinuity and extraluminal gallstones, and indirect findings including pericholecystic fluid, gallbladder wall thickening, fat stranding, and ascites (2).

In the present case, the most significant diagnostic finding was the presence of extraluminal gallstones, a direct sign of perforation (2). What makes this case particularly noteworthy is the availability of prior CT imaging, which demonstrated the same gallstones previously located within the gallbladder lumen. This comparison provided strong evidence of stone migration into the peritoneal cavity and substantially increased diagnostic confidence.

Conclusion

Early diagnosis and prompt treatment of gallbladder perforation are essential to reduce morbidity and mortality. This case highlights the important role of CT in the diagnosis of gallbladder perforation, particularly the added value of comparison with prior imaging in increasing diagnostic confidence when characteristic findings such as extraluminal gallstones are identified.

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Sporadic insulinoma – rare case presentation

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Background: Insulinomas are a rare type of functional neuroendocrine neoplasm which develop from the pancreatic islet cells. The incidence is roughly 1–4 people per million; they can occur sporadically or in conjunction with MEN-1 syndrome. Sporadic insulinomas are usually benign tumors with peak incidence between 5th–6th decade of life, and a slight female predominance. They typically present with the Whipple's triad – symptoms consistent with hypoglycemia, a low documented plasma glucose concentration (≤ 2.8 mmol/L), and relief of symptoms after glucose administration. The mainstay of the diagnosis is biochemical confirmation of endogenous hyperinsulinemic hypoglycemia – decreased plasma glucose, and elevated insulin and C-peptide levels. **Case presentation:** We demonstrate a rare case of a 72-year-old woman presenting with a 4-year history of constipation, epigastric pain after meals, weight loss, and insomnia. Within the last 2 years, she has developed recurring episodes of hypoglycemia, dizziness, sweating, and palpitations. While initially she presented with a normal random glucose and a negative CT scan, subsequent testing with MRI showed a lesion on the head of the pancreas. Further testing confirmed hypoglycemia during fasting with inappropriately elevated insulin and C-peptide levels. **Conclusion:** The irregularities in the laboratory testing and imaging, along with certain inconsistencies in clinical presentation, highlight the challenges faced when attempting to diagnose a rare presentation of a rare disease. We consider this publication useful in depicting a more complex presentation of this disease, and we hope that it highlights the importance of further investigation in cases of prolonged hypoglycemia.
Keywords: *insulinoma, hypoglycemia, endocrinology*

Introduction

Pancreatic neuroendocrine tumors (PanNETs) are a rare type of neoplasm with an approximate annual incidence of 0.8 per 100,000. Among all PanNETs, insulinomas are the most common functional type arising from the pancreatic islet cells.[1] The incidence is roughly 1–4 people per million, and they represent just 1–2% of pancreatic neoplasms[2]. They can occur sporadically or in conjunction with four hereditary syndromes: multiple endocrine neoplasia (MEN-1) syndrome, neurofibromatosis type 1 (NF1), Von Hippel–Lindau (VHL) disease, and tuberous sclerosis (TSC). Sporadic insulinomas are usually benign tumors with peak incidence between the 5th and 6th decades of life, and a slight female predominance[2]. Insulinomas typically secrete insulin in an episodic manner regardless of blood glucose levels. Patients present with episodes of hypoglycemia, which have been classified into two main categories: neuroglycopenic (diplopia, blurred vision, altered mental status, abnormal behavior, amnesia, and seizures) and adrenergic (sweating, anxiety, palpitations, weakness, dizziness, tremors, hunger, and nausea)[1]. Additionally, most of them experience hyperphagia and subsequent weight gain[3]. Patients present with fasting hypoglycemia in about 73% of cases, and about 20% percent of patients present with both fasting and postprandial hypoglycemia[4].

The classical diagnosis is typically present with the Whipple's triad – symptoms consistent with hypoglycemia, a low documented plasma glucose concentration (≤ 2.8 mmol/L), and relief of symptoms after glucose administration[5]. The mainstay of the diagnosis is biochemical confirmation of endogenous hyperinsulinemic hypoglycemia – decreased plasma glucose, and elevated insulin and C-peptide levels[6]. Once the diagnosis has been confirmed, noninvasive and invasive localization studies with different sensitivity rates are utilized to improve operative success. The most commonly used

techniques include 3-phase CT (60–80%), MRI (85–90%), and endoscopic ultrasound (EUS) (75–90%)[7]. Because insulin secretion may be intermittent and symptoms are often nonspecific, the diagnosis of insulinoma may be delayed. Atypical clinical features, equivocal laboratory values, and initially negative imaging studies may further complicate recognition of the disease.

Case presentation

We demonstrate a rare case of a 72-year-old woman referred to the Endocrinology clinic for a current metabolic and hormonal evaluation in relation to persistent hypoglycemia with a duration of more than 2 years. She reports weight loss of 10 kg in 7 months, insomnia, and depressive episodes. She has a 4-year history of constipation, difficulty with defecation, and heaviness and pain in the epigastric region after eating. Within the last 2 years, she has developed recurring episodes of hypoglycemia, dizziness, sweating, and palpitations. Her BMI was 19.7 kg/m², and she was well oriented in time, place, and person. She has no previous surgical history, does not smoke, and does not consume alcohol. In September 2023, she was hospitalized in the Endocrinology clinic, where hyperinsulinemic hypoglycemia was considered unlikely. A contrast-enhanced CT scan was negative for any tumor formations. In January 2024, upper and lower gastrointestinal endoscopy demonstrated gastritis and duodenitis, which did not adequately explain her symptoms. Upon subsequent hospitalizations, an abdominal contrast-enhanced MRI revealed a hypervascular lesion of the head of the pancreas extending to the descending part of the duodenum and measuring 15/11 mm, suggestive of an insulinoma. No enlarged peripheral lymph nodes were identified. ECG showed sinus rhythm and an old left bundle branch block. Laboratory results obtained during the current hospitalization are presented in Table 1.

Table 1. Laboratory tests during hospitalization

Parameter (reference range)	**Value**
Hemoglobin (110–160 g/L)	144.0
Eritrocytes (3.5–5.5 x10^{12}/L)	4.44
White blood cell (3.5–10 x10^{9}/L)	6.06
Platelets (140–400×10^{9}/L)	226.0
C-reactive protein (0.0–6.0 mg/L)	0.7
Sodium (136–151 mmol/L)	145.0
Potassium (3.5–5.6 mmol/L)	3.8
Chloride (97–108 mmol/L)	104.0
Calcium (2.02–2.60 mmol/L)	2.4
Phosphorus (0.8–1.5 mmol/L)	1.1
PTH (0.7–1.06 mmol/L)	49.9
Albumin (35–52 g/L)	40.3
Alanine transaminase (0–45 U/L)	11
Aspartate aminotransferase (0–38 U/l)	22
HbA1C (4.0–6.4%)	3.9
TSH (0.34–5.6)	0.462
FT3 (3.8–6.0)	4.48
FT4 (9.0–23.0)	13.03
Anti TPO Ab (0.05–10.0)	1058.6
ACTH (6–48)	35.8

During a supervised fasting test, plasma glucose fell to 1.72 (3.5–6.2) mmol/L after 3 hours, with a simultaneously measured insulin level of 7.6 (1.1–17.0) mIU/L and C-peptide level of 1.38 (0.93–3.9) ng/mL. Although both insulin and C-peptide values were within the laboratory reference range, they were inappropriately non-suppressed in the setting of marked hypoglycemia, supporting the diagnosis of endogenous hyperinsulinemic hypoglycemia. The patient was advised to follow a dietary regimen consisting of frequent meals and avoidance of rapidly absorbable carbohydrates, with preference for low-glycemic-index foods. Because diazoxide was unavailable in our country, prednisolone was initiated as a bridging therapy before surgery. The pancreatic lesion

was surgically enucleated. Histopathological examination confirmed a well-differentiated neuroendocrine tumor, consistent with insulinoma. Postoperatively, her blood glucose levels remained within the normal range, and her presenting symptoms resolved.

Discussion

Insulinomas are rare functional pancreatic neuroendocrine tumors characterized by inappropriate insulin secretion despite low plasma glucose concentrations. Under physiological conditions, insulin secretion is suppressed during hypoglycemia. In contrast, insulinomas continue to secrete insulin independently of glucose levels, leading to increased peripheral glucose uptake, inhibition of hepatic gluconeogenesis and glycogenolysis, and ultimately recurrent hypoglycemia[8]. Biochemical evaluation is central to the diagnosis, most commonly performed using a supervised fasting test. In the present case, although insulin and C-peptide were within laboratory reference ranges, they were inappropriate in the setting of significant hypoglycemia, thereby supporting endogenous hyperinsulinemic hypoglycemia.

The differential diagnosis of hypoglycemia is broad and requires a systematic approach, particularly in atypical presentations. Causes can be broadly divided into insulin-mediated and non–insulin-mediated etiologies. Insulin-mediated hypoglycemia includes exogenous insulin administration, sulfonylurea or meglitinide use, and endogenous hyperinsulinism such as insulinoma or non-insulinoma pancreatogenous hypoglycemia syndrome[10]. Measurement of insulin and C-peptide levels is essential in distinguishing these conditions. In exogenous insulin administration, insulin levels are elevated while C-peptide is suppressed, whereas in endogenous hyperinsulinism, both are detectable[9].

Non–insulin-mediated causes include critical illnesses such as hepatic, renal, or cardiac failure, in which impaired gluconeogenesis contributes to hypoglycemia. Endocrine disorders, particularly adrenal insufficiency and hypopituitarism, should also be considered, as cortisol deficiency disrupts glucose homeostasis. Sepsis, alcohol-related hypoglycemia, and non-islet cell tumor hypoglycemia mediated by insulin-like growth factor 2 (IGF-2) are additional recognized causes. In our patient, the absence of systemic illness, normal cortisol and ACTH levels, and lack of exposure to hypoglycemic agents made these etiologies unlikely[9].

An important consideration in patients diagnosed with insulinoma is the possibility of an underlying hereditary syndrome, most notably multiple endocrine neoplasia type 1 (MEN1). MEN1 is an autosomal dominant disorder caused by mutations in the MEN1 gene and is characterized by tumors of the parathyroid glands, pancreatic neuroendocrine tumors, and pituitary adenomas [11]. Insulinomas associated with MEN1 tend to occur at a younger age, are more frequently multiple, and may coexist with other pancreatic neuroendocrine tumors. In contrast, sporadic insulinomas are typically solitary and present later in life[9]. In the present case, the patient's advanced age, solitary pancreatic lesion, and absence of clinical or biochemical evidence of hyperparathyroidism or pituitary disease make MEN1 unlikely, supporting a sporadic etiology.

Tumor localization represents another diagnostic challenge. Imaging modalities such as contrast-enhanced computed tomography and magnetic resonance imaging have variable sensitivity, particularly for small lesions[12]. In this case, the initial CT scan was negative, whereas the subsequent MRI identified a small hypervascular lesion in the pancreatic head. This underscores the

importance of repeated or complementary imaging when clinical suspicion remains high despite inconclusive initial findings. Several features of this case contributed to diagnostic difficulty. First, the patient presented with weight loss rather than the more typical weight gain associated with compensatory hyperphagia. Second, her symptoms included chronic constipation and postprandial epigastric discomfort, which are not characteristic of insulinoma and may have suggested a gastrointestinal etiology. Third, initial biochemical and imaging findings were inconclusive, further delaying diagnosis.

Surgical resection remains the definitive treatment for insulinoma and is curative in the majority of cases[8]. In this patient, surgical enucleation resulted in normalization of blood glucose levels and complete resolution of symptoms, confirming the diagnosis.

Conclusion

The irregularities in the laboratory testing and imaging, along with certain inconsistencies in clinical presentation, highlight the challenges faced when attempting to diagnose a rare presentation of a rare disease. We consider this publication useful in depicting a more complex presentation of this disease, and we hope that it highlights the importance of further investigation in cases of prolonged hypoglycemia.

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Clinical case report: a rare UMOD variant (c.359G>A) in a 37-year-old patient with hyperuricemic nephropathy

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Background: Familial juvenile hyperuricemic nephropathy is part of the spectrum of autosomal dominant tubulointerstitial kidney diseases (ADTKD), characterized by early-onset hyperuricemia, gout, and progressive chronic kidney disease. Mutations in the UMOD gene represent the most common genetic cause of ADTKD. **Case presentation:** We present a 37-year-old female patient with a history of gout and pseudogout, with the first clinical manifestations occurring in 2022. Laboratory investigations revealed hyperuricemia, low-grade proteinuria, and reduced estimated glomerular filtration rate, consistent with chronic kidney disease. Given the positive family history and clinical presentation, a hereditary nephropathy was suspected. **Methods:** Genetic testing was performed using next-generation sequencing with the nephritis FLEX panel (Blueprint Genetics), targeting genes associated with hereditary kidney diseases. **Results:** A heterozygous missense variant in the UMOD gene, c.359G>A (p.Cys120Tyr), was identified. The variant affects a highly conserved cysteine residue within an EGF-like calcium-binding domain of the uromodulin protein. In silico analyses predict a damaging effect on protein structure and function. The variant is absent from population databases and has been previously reported only once in the literature, supporting its classification as likely pathogenic. **Conclusion:** This case highlights the importance of considering UMOD-associated ADTKD in patients presenting with early-onset gout, hyperuricemia, and progressive chronic kidney disease. Genetic testing plays a crucial role in establishing the diagnosis, guiding patient management, and enabling appropriate family counseling.

Keywords: *Familial juvenile hyperuricemic nephropathy, ADTKD-UMOD, UMOD gene mutation, hyperuricemia/early-onset gout, chronic tubulointerstitial kidney disease*

1. Introduction to autosomal dominant tubulointerstitial kidney disease (ADTKD)

Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD) represents a critical diagnostic category in clinical nephrology, encompassing a group of rare monogenic disorders characterized by progressive renal decline. The clinical presentation is notoriously insidious, typically featuring a "bland" urinary sediment and minimal proteinuria, which frequently leads to the underdiagnosis or late-stage identification of chronic kidney disease (CKD). Historically, these conditions were categorized under various eponymous terms, such as Medullary Cystic Kidney Disease type 2 (MCKD2), Familial Juvenile Hyperuricemic Nephropathy (FJHN), and Glomerulocystic Kidney Disease (GCKD); however, they are now unified under the ADTKD umbrella, classified by the underlying genetic driver.

The molecular architecture of ADTKD is primarily defined by mutations in the *UMOD*, *MUC1*, and *REN* genes. Among these, pathogenic variants in the *UMOD* gene – encoding uromodulin, also known as the Tamm–Horsfall protein are the most prevalent. Uromodulin is the most abundant protein in normal human urine, synthesized exclusively by the epithelial cells of the thick ascending limb (TAL) of the loop of Henle. In renal physiology, uromodulin is indispensable; it regulates salt and water transport via the modulation of sodium-potassium transporters, provides innate immunological defense against urinary tract infections by preventing bacterial adhesion, and inhibits the crystallization of calcium oxalate. A disruption in this protein's structure not only impairs these protective functions but initiates a cytotoxic cascade within the tubular epithelium. This report details the clinical investigation of a rare *UMOD* variant and its systemic manifestations in a young adult patient.

2. Clinical case presentation: 37-year-old female patient

A high index of clinical suspicion is required when metabolic derangements, such as early-onset gout, coincide with signs of renal insufficiency. Given that hyperuricemia can function as both a driver and a marker of renal pathology, distinguishing between primary metabolic gout and a monogenic tubulointerstitial disorder is essential for appropriate longitudinal management.

The patient, a 37-year-old female, presented in 2022 with a clinical history of recurrent gout and gouty arthritis. Laboratory evaluation revealed a constellation of findings suggestive of ADTKD-UMOD, including hyposthenuria and metabolic markers at the threshold of clinical concern.

Summary of diagnostic markers

Clinical parameter	Finding/Value
Symptomatology	Gout and Gouty Arthritis (Onset 2022), Polyuria, Nocturia
Urinary Analysis	Hyposthenuria (Low Specific Gravity/Osmolarity), Low-grade proteinuria (< 1 g/24 h)
Urinary Sediment	Bland (Absence of hematuria, dysmorphic red cells, or casts)
Serum Biochemistry	Hyperuricemia; Upper-limit serum creatinine and urea levels
Renal Function	Reduced eGFR (Chronic Kidney Disease)

The “bland” nature of the urinary sediment was a diagnostic clue. The absence of hematuria and significant albuminuria effectively ruled out primary glomerular disease, directing the diagnostic focus toward the renal interstitium and tubular basement membranes. Furthermore, the presence of polyuria and nocturia underscored a defect in urinary concentration typical of TAL dysfunction. Given the relatively young age of onset and a positive family history for renal disease, the patient was referred to the Flex Bulgarian Genetics Laboratory for targeted molecular analysis.

3. Genetic analysis and variant characterization (c.359G>A)

The integration of Next-Generation Sequencing (NGS) has become the gold standard for resolving the etiology of unexplained CKD. Comprehensive sequencing analysis utilizing the FLEX Blueprint Genetics (BpG) nephrolithiasis panel identified a heterozygous missense variant: **UMOD c.359G>A, p.(Cys120Tyr)**.

The pathogenicity of the **p.(Cys120Tyr)** variant is established through several converging lines of evidence:

- **Locus:** The mutation is situated within the highly sensitive EGF-like calcium-binding domain of the uromodulin protein.
- **Physicochemical impact:** The substitution involves the replacement of Cysteine – a sulfur-containing amino acid critical for disulfide bridge formation – with Tyrosine, a large aromatic amino acid. This results in a significant physicochemical discrepancy, evidenced by a Grantham score of 94, indicating a high probability of structural damage.
- **Population data:** The variant is absent from the gnomAD database (spanning ~120,000 exomes and 15,000 genomes), confirming its extreme rarity in the general population.
- **Conservation and paralogous data:** The affected residue is highly conserved across species. Furthermore, other substitutions at this exact codon – **p.(Cys120Arg)** and **p.(Cys120Gly)** – have been previously linked to UMOD-associated phenotypes, confirming that codon 120 is a critical functional node for protein stability.

Based on these findings, the variant is classified as “likely pathogenic”, providing a definitive genetic explanation for the patient's hyperuricemic nephropathy.

4. Molecular pathogenesis: ER stress and proteotoxicity

The transition from a single nucleotide substitution to progressive organ failure is driven by the intracellular accumulation of misfolded proteins. In ADTKD-UMOD, the loss of a Cysteine residue at position 120 disrupts the intramolecular disulfide bonds essential for the stability of the uromodulin D8C domain.

This structural destabilization triggers a pathological cascade:

1. **Protein misfolding and ER retention:** The mutant uromodulin fails to achieve its native conformation and is consequently retained within the endoplasmic reticulum (ER) of the TAL epithelial cells.
2. **ER stress induction:** The massive accumulation of these aberrant, polymorphic, unstructured proteins induces chronic ER stress and activates the unfolded protein response (UPR).
3. **Proteotoxicity and apoptosis:** Persistent ER stress leads to accelerated tubular cell apoptosis. As TAL cells die, they are replaced by interstitial fibrosis and lamellation of the tubular basement membrane.
4. **Secondary metabolic derangement:** The failure to correctly traffic uromodulin to the apical membrane reduces functional protein levels in the urine. This leads to a decreased expression of ion transporters, which triggers a compensatory increase in sodium reabsorption in the proximal tubule. This process results in a secondary, passive increase in proximal uric acid reabsorption, explaining why early-onset hyperuricemia and reduced fractional excretion of urate often precede the decline in GFR.

5. Comparative analysis: the international reference case

Validating the clinical significance of rare variants requires the utilization of international genetic databases. At the time of diagnosis, the **c.359G>A** variant was nearly unique globally, emphasizing the importance of gene-phenotype correlation in confirming pathogenicity.

The patient's clinical presentation is remarkably congruent with the only other documented case: a 28-year-old Pakistani female. A comparative review of these cases reveals a consistent phenotype:

- Shared genotype: Heterozygous c.359G>A variant.
- **Metabolic onset:** Early-onset hyperuricemia and clinical gout manifesting in the third decade of life.
- **Renal progression:** Slowly progressive CKD with tubulointerstitial characteristics and impaired concentration capacity.

This correlation solidifies the classification of **c.359G>A** as a severe pathogenic variant. Given the autosomal dominant inheritance pattern, this diagnosis carries profound implications for the patient's kindred, as each first-degree relative possesses a 50% risk of harboring the mutation.

6. Management strategy and clinical implications

Strategic management of ADTKD-UMOD focuses on the prevention of rapid progression to End-Stage Renal Disease (ESRD). Evidence suggests that the prognostic trajectory is heavily dependent on the timing of intervention. Specifically, clinical data indicates that:

- Patients with a serum creatinine **< 200 µmol/l** at diagnosis can maintain stable renal function for **6 years** or longer with optimal therapy.
- Patients with normal creatinine levels at the start of treatment have shown stability for up to **20 years**.
- Conversely, once creatinine exceeds **200 µmol/l**, progression to ESRD, dialysis,

and transplantation is often rapid and poorly responsive to intervention.

The following comprehensive management plan is recommended:

- **Pharmacological intervention:** Immediate initiation of **Allopurinol** (or Benzobromarone) to manage hyperuricemia. Research confirms that Allopurinol significantly reduces the morbidity and mortality associated with renal failure in these patients.
- **Nutritional therapy:** Adoption of a low-purine and caffeine-free diet to reduce the metabolic burden.
- **Longitudinal monitoring:** Regular assessment of eGFR, uric acid clearance, and creatinine levels to monitor the rate of nephron loss.
- **Family screening:** Urgent genetic counseling and clinical screening for all first-degree relatives, particularly children and young adults, to facilitate early diagnosis.

7. Conclusion

In summary, this case highlights the necessity of integrating genetic sequencing into the nephrological workflow for patients with unexplained CKD and early-onset gout. Identifying the **c.359G>A** variant allows for a targeted, evidence-based approach that may significantly extend the patient's renal survival and improve the long-term outlook for the entire family.

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Effect of obstructive sleep apnea on the rhythmogenic risk in patients with newly diagnosed metabolic syndrome

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Obstructive sleep apnea often accompanies metabolic syndrome and is an additional risk factor for arrhythmias. According to current ideas, there is a bidirectional relationship between the two. The **aim** of the present study is to investigate the proarrhythmogenicity of polysonographic indicators in newly diagnosed metabolic syndrome. **Material and methods:** 71 patients with newly diagnosed metabolic syndrome, aged 35–55 years, were studied. All patients underwent a 48-hour Holter ECG recording. Based on the latter, they were divided into two groups – with a high arrhythmogenic load – 38 patients (53.5%) (includes supraventricular or ventricular tachycardia, atrial fibrillation/flutter, ventricular extrasystoles over 10%, frequent supraventricular extrasystoles > 500/24 h) and with low arrhythmogenic load – 33 patients – 46.5% (includes no significant rhythm disturbances). In all patients, an electrocardiogram was performed at baseline with a 12-channel ECG. Tpe and QT intervals are measured automatically at the Bulgarian Academy of Sciences. Polysonography was used to examine parameters – apnea-hypnoea index (AHI), desaturation index (ODI). **Results:** Of the patients studied, only 1 was without sleep apnea. There is no statistical significance for AHI between the two groups. ($p > 0.05$) In the group with low arrhythmogenicity, there is an almost similar representation of the three types of sleep apnea severity, while in the group with high arrhythmogenicity, severe sleep apnea is represented more than twice as often as mild, and moderate occupies an intermediate position between mild and moderate sleep apnea. No statistical difference in ODI was observed in the two groups of patients studied. ($p > 0.05$) **Conclusion:** AHI and ODI are not proarrhythmogenic markers in newly diagnosed metabolic syndrome.

Keywords: *metabolic syndrome, polysonography, apnea-hypnotic index, desaturation index, proarrhythmogenicity*

Introduction

Obstructive sleep apnea often accompanies metabolics yndrome and is an additional risk factor for arrhythmias. According to current research, there is a bidirectional relationship between both disorders. (1–3) Cardiomyocyte changes induced by metabolic and proinflammatory factors impair repolarization, exacerbate the heterogeneity of the transmural dispersion of repolarization. (4–9)

Aim

The aim of the present study is to investigate the association between polysomnographic markers and proarrhythmogenic risk in newly diagnosed patients with metabolic syndrome.

Material and methods

Were cruited 71 patients with newly diagnosed metabolic syndrome, aged 35–55 years, mean age 45. All patients underwent a 48-hour Holter ECG recording (Fig. 1) and single-night polysomnography (PSG). (Fig. 2)

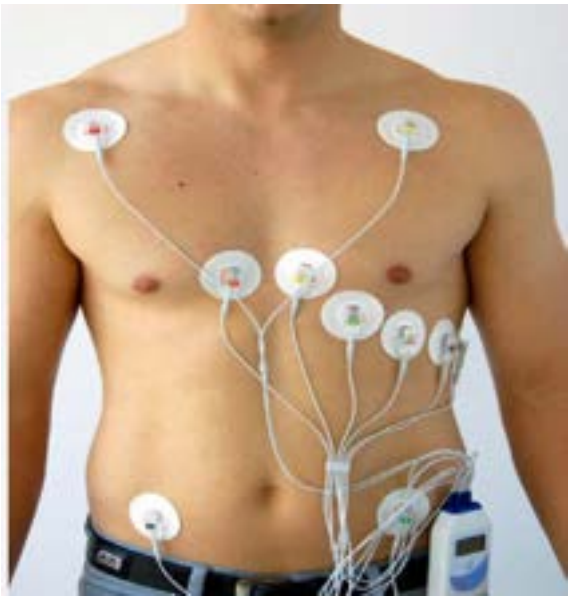

Figure 1. 48-hour Holter ECG recording

Figure 2. Single-night polysomnography

Based on the latter, they were divided into two groups – with a high arrhythmogenic load – 38 patients (53.5%) (includes supraventricular or ventricular tachycardia, atrial fibrillation/ flutter, ventricular extrasystoles over 10%, frequent supraventricular extrasystoles > 500/24 h) and with low arrhythmogenic load – 33 patients – 46.5% (includes no significant rhythm disturbances). In all patients, an electrocardiogram was performed at baseline with a 12-channel ECG.

Results

The studied group had an uneven distribution so the data was analysed with the use of Mann Whitney test. Between the two groups there was no statistically significant difference in the values for AHI index and ODI. (Table 1)

Even when split in to two groups with severe and non-severe AHI, results failed to show statistical significance. (Figure 3)

Table 1. Comparison between AHI and ODI between both groups

Variable	Group	N	Mean ± SEM	Median (IQR)	t-value	P-value
AHI Index	LA	33	29.96 ± 4.54	22.60 (33.30)	1.557	0.124
	HA	35	40.91 ± 5.32	209.10 (41.30)		
ODI	LA	33	30.33 ± 4.45	21.80 (38.70)	1.428	0.158
	HA	35	40.36 ± 5.37	29.50 (43.60)		

AHI, apnea-hypopnea index; ODI, oxygen desaturation index;
LA, low arrhythmogenicity; HA, high arrhythmogenicity

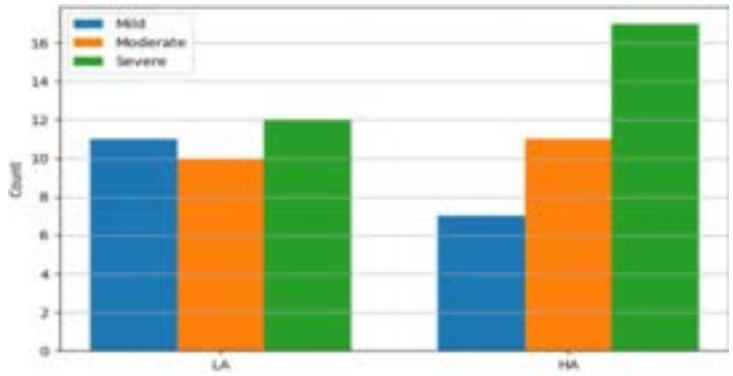

Figure 3. AHI in both groups according to severity

Table 2. Comparing groups when regrouping

Variable	Category	Low Arrhythmogenicity	High Arrhythmogenicity	Total
AHI	**Mild + Moderate (Count)**	**21**	**18**	**39**
	(%)	53.8%	46.2%	100.0%
	Severe (Count)	12	17	29
	(%)	41.4%	58.6%	100.0%
All	**Count**	**33**	**35**	**68**
	%	48.5%	51.5%	100.0%

Fisher's exact test, P = 0.337

In the low arrhythmogenicity group, there is an almost similar representation of the three types of sleep apnea severity (1 – mild, 2 – moderate, 3 – severe), while in the high arrhythmogenicity group, severe sleep apnea is represented more than twice as much as mild sleep apnea, and moderate sleep apnea occupies an intermediate position between mild and moderate sleep apnea. (Figure 3 and Table 2) (1–5)

Another indicator of the severity of the disorders observed in patients with OSA is the desaturation index, which correlates with the severity of apnoea pauses. In the two groups of patients studied by us, no statistically significant difference in the values of the desaturation index was observed. (Table 1) This may be due to the selection of patients and the lack of underlying ischemic heart disease or heart failure, two conditions that are characterized by high sensitivity of the myocardium to hypoxaemia. (6–10)

Conclusion

Our results suggest that AHI and ODI may not be useful markers for determining the arrhythmogenic burden in patients with metabolic syndrome and obstructive sleep apnea.

Acknowledgments

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Periodic limb movements during sleep as a proarrhythmogenic marker in newly diagnosed metabolic syndrome

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Periodic limb movements during sleep (PLMS) are a frequent finding on polysomnography. Although PLMS are associated with transient increases in sympathetic tone, their clinical significance as an arrhythmogenic risk factor remains debatable. The **aim** of the present study was to investigate the association between PLMS and arrhythmogenic risk in newly diagnosed patients with metabolic syndrome. **Material and methods:** Seventy-one patients with newly diagnosed metabolic syndrome, aged 35–55 years, were studied. All participants underwent a 48-hour Holter ECG monitoring and were stratified into two groups – high arrhythmogenic load (n = 38, 53.5%) and low arrhythmogenic load (n = 33, 46.5%). High arrhythmogenic load was defined by the presence of supraventricular or ventricular tachycardia, atrial fibrillation/flutter, ventricular extrasystoles over 10%, and frequent supraventricular extrasystoles > 500/24 h. All participants underwent overnight polysomnography, with manual scoring performed in concordance with the American Academy of Sleep Medicine (AASM) criteria. The PLMS index was significantly higher in the high arrhythmogenic load group compared with the low arrhythmogenic load group (31.17 ± 6.76 vs 13.58 ± 2.85, mean ± SEM, p = 0.021) The presence of a PLMS index > 15 was associated with an almost 3-fold increase in the arrhythmogenic risk. odds ration [OR] = 2.875 95% confidence interval [CI]:1.033–8.00). **Conclusion:** A PLMS index ≥ 15 is a significant risk factor for cardiac rhythm disturbances in newly diagnosed metabolic syndrome and may identify individuals who would benefit from Holter ECG screening.

Keywords: *metabolic syndrome, Polysomnography, apnea-hypnotic index, desaturation index, periodic limb movements during sleep*

Introduction

Obstructive sleep apnea accompanies metabolic syndrome and is associated with arrhythmias. In clinical practice, the latter are often detectedlate. There is a growing need for accessible and clinically informative markers for the assessment of proarrhythmic burden, with periodic limb movement index (PLMD) represent in ga potentially valua bleand under recognized indicator. (1–10)

Aim

The aim of the present study was to evaluate the association between polysomnographic parameters, particularly periodic limb movements during sleep, and proarrhytmogenic burden in newly diagnosed patients with metabolic syndrome.

Material and methods

Were cruited 71 patients with newly diagnosed metabolic syndrome, aged 35–55 years, mean age 45. All patients underwent a 48-hour Holter ECG recording and single-night polysomnography. (Fig. 1) **Polysomnography parameters studied – apnea-hypopnea index (AHI), oxygen desaturation index (ODI), PLMS index.**

Based on the latter, they were divided into two groups – with a high arrhythmogenic load – 38 patients (53.5%) (includes supraventricular or ventricular tachycardia, atrial fibrillation/ flutter, ventricular extrasystoles over 10%, frequent supraventricular extrasystoles > 500/24 h) and with low arrhythmogenic load – 33 patients – 46.5% (includes no significant rhythm disturbances). In all patients, an electrocardiogram

was performed at baseline with a 12-channel ECG.

Figure 1. Single-night polysomnography

Results

The studied group had anuneven distribution so the data was analysed with the use of MannWhitney test. Between the three analysed parameters only PLMS had a statistically significant difference between the two groups, as shown in table 1.

When apply in ga cut-ff value of 15 for PLMS, data showed that the high arrhythmogenic group had statistically significant higher values than the lower rhythmogenicty group. (Table 2) The latter signified at hree fold increase in the arrhythmogenic burden for PLMS values > 15.

Table 1. Comparison of AHI INDEX, ODI, and PLMS index between both groups

Groups	N	Mean ± SEM	Median (IQR)	t	P-value
AHI INDEX	LA	29.96±4.54	22.60 (33.30)	1.557	0.124
	HA	40.91±5.32	209.10 (41.30)	1.557	0.124
ODI	LA	30.33±4.45	21.80 (38.70)	1.428	0.158
	HA	40.36±5.37	29.50 (43.60)	1.428	0.158
PLMS	LA	13.58±2.85	6.40 (21.90)	2.40	0.021
	HA	31.17±6.76	17.00 (50.50)	2.40	0.021

AHI – apnea-hypopnea index; ODI – oxygen desaturation index; PLMS – periodic limb movement in sleep; LA – low arrhythmogenicity; HA – high arrhythmogenicity

Table 2. Different levels of PLMS index in both group

Groups		Groups		All
		Low arrhythmogenicity	High arrhytmogenicity	
PLMS index	<15 Count	23 59.0%	16 41.0%	39 100.0%
≥15	<15 Count	9 33.3%	18 66.7%	27 100.0%
All	Count %	32 48.5%	34 51.5%	66 100.0%
		Fisher's exact test, P=0.049		

Discussion

The data from the frequency distribution analysis presented in Table 33 show that PLMS can be used as a marker to differentiate patients with metabolic syndrome and with low and high arrhythmogenicity. A statistically significant difference in the distribution depending on PLMS was found in the studied groups with low and high arrhythmogenic load (Fisher's exact test, $P = 0.049$). The Odds Ratio estimate is 2.875 95% CI [1.033; 8.00]. This result indicates that the chance of patients with PLMS < 15 to have low arrhythmogenic load is almost three times higher compared to patients with PLMS ≥ 15. (1–10)

Conclusion

Our finding suggest that elevated PLMS may represent a useful marker of increased arrhythmogenic risk and warrant further investigation in larger prospective studies.

Acknowledgments

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Extracorporeal renal replacement in sepsis: hemodynamic and pharmacokinetic challenges

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In patients with sepsis and renal failure, hemodiafiltration is the preferred method for extracorporeal renal replacement therapy compared to intermittent dialysis, which has more frequent hemodynamic complications. In critical cases, coagulopathy is observed as a result of an inflammatory cascade, endothelial damage, and disorders in platelet function. The careful selection of an anticoagulant is an important part of the complex treatment of obstructive renal failure. Unfractionated heparin is most often used, as it has a short half-life and the possibility of rapid correction. However, its use is limited due to the risk of accumulation in impaired clearance. Citrate anticoagulation is less common in these conditions, as it requires strict calcium monitoring and is problematic in liver dysfunction, which often accompanies patients with septic complications. Significant changes in pharmacokinetics are observed, such as increased volume of distribution and vascular permeability and unstable hemodynamics, which lead to unpredictable plasma levels of medications, and hemofiltration accelerates the elimination of hydrophilic agents. In patients on vasopressor therapy, it is necessary to introduce strategies to optimize the volume and dosage regimen of vasopressors, the blood flow rate used, the dialysis flow, the filtration fraction, the regimen used, the dialysis temperature and the membrane before including hemodiafiltration, since the altered vascular tone and hypoperfusion affect the distribution and effectiveness of vasopressors. In the absence of universal and applicable guidelines, the therapeutic choice should be individualized according to the risk of bleeding, the degree of hemodynamic instability and the characteristics of hemofiltration.

Keywords: *sepsis, hemodiafiltration, extracorporeal renal replacement therapy, pharmacokinetics*

Introduction

According to the Sepsis-3 consensus definition, the condition is characterized as a heterogeneous syndrome of life-threatening organ dysfunction resulting from a dysregulated host immune response to a pathogenic agent. Central to the initiation of the pathological process is the recognition of molecular patterns by the innate immune system. The main participants in this inflammatory cascade are:

- PAMPs (Pathogen-Associated Molecular Patterns): Exogenous signals such as lipopolysaccharides (LPS) from gram-negative bacteria or peptidoglycans from gram-positive organisms.
- DAMPs (Damage-Associated Molecular Patterns): Endogenous "danger signals" (e.g. HMGB1 proteins, mitochondrial DNA) released from damaged or necrotic host cells as a result of tissue ischemia or direct cytotoxicity.

These mediators act as ligands for Toll-like receptors (primarily TLR2 and TLR4) localized on the membranes of endothelial cells and tubular epithelium. The ligand-receptor binding activates intracellular signaling pathways (such as NF-κB), which induce transcription of pro-inflammatory cytokines, leading to a systemic intensive inflammatory cascade [1].

Endothelial activation under the influence of the cytokine storm leads to pathological structural changes in the protective glycocalyx – the delicate network of glycoproteins and proteoglycans covering the luminal surface of blood vessels. Its degradation (shedding) compromises the vascular barrier, leading to:

- Pathological capillary permeability: Increased transudate of fluids and macromolecules into the interstitial space,

generating diffuse edema and impairing oxygen diffusion.

- Loss of anticoagulant properties: The endothelium transitions from an antithrombotic to a pro-inflammatory status, initiating the formation of microthrombi.

A critical aspect in the pathophysiology of S-AKI (sepsis-induced acute kidney injury) is the so-called hemodynamic dissociation. While systemic blood pressure may be maintained within reference ranges through vasopressors, severe disorganization is observed at the microcirculatory level. Processes of leukocyte adhesion (margination and diapedesis) and local microthrombosis block some capillaries, while others remain pathologically dilated.

The result is a heterogeneous capillary flow in which functional capillary units are interspersed between zones of stasis and ischemia. This mechanism explains the presence of tissue hypoxia and cellular distress even under clinically apparently stable hemodynamic parameters [2].

Collectively, these mechanisms, along with the pathological metabolic reprogramming of cells (switching to aerobic glycolysis or “Warburg metabolism”) in order to prevent programmed cell death, lay the foundation for sepsis-associated acute kidney injury (SA-AKI) – the most common complication in critically ill patients. The leading clinical problems are hemodynamic instability (septic shock) and progressive multi-organ dysfunction, which drastically alter the fluid-electrolyte balance and homeostasis. Aggressive fluid resuscitation and changes in protein binding further disrupt the pharmacokinetics of vital medications, rendering standard dosing ineffective or toxic [3][4].

Aim

The aim of this paper is to conduct a comparative analysis of hemodiafiltration (HDF) and intermittent hemodialysis (IHD) as methods of renal replacement therapy (RRT), examining their influence on hemodynamic stability and pharmacokinetic changes in critically ill patients with sepsis.

Materials and methods

Renal replacement therapy in SA-AKI is based on two fundamental physicochemical principles:

Diffusion, which in essence is the movement of small molecules along a concentration gradient across a semipermeable membrane – the primary mechanism in IHD, effective for low-molecular-weight toxins such as urea and creatinine.

Convection, which is the transport of substances via solvent drag (movement of fluid under hydrostatic pressure), effective for medium-molecular-weight toxins (up to ~50,000 Da) and predominant in hemofiltration (HF).

Hemodiafiltration (HDF/CVVHDF) integrates both principles, achieving maximum clearance capacity for a broad spectrum of toxins. Continuous methods (CRRT) include CVVH (pure convection), CVVHD (continuous diffusion), and CVVHDF. Acute kidney injury is classified according to KDIGO criteria into three stages, with stage 3 including the need for RRT and anuria for $\geq$ 12 hours. This analysis reviews data from large-scale studies (ESHOL, CONVINCE, CONTRAST) to evaluate the clinical efficacy of high-volume online HDF.

Mathematical modelling is a critical methodology to describe the physicochemical principles. Pharmacokinetic models are described via the ADME principle – absorption, distribution, metabolization, elimination of the drug in an appropriate matrix. The models are divided into two main groups – individual and population. The individual models require rich concentration-time data, and they are usually used for cases with non-typical rapid change in the pharmacokinetic

parameters. The population models represent a method, based on Model-Informed Drug Development, resulting from the intrinsic and extrinsic factors. They are applied for the evaluation of the Exposure-Response, Concentration-QT, In vitro-In vivo Correlation, Allometric scaling, etc. These models are essential for the prediction of the pharmacokinetics across populations. The combination with pharmacodynamic data supports the assessment of therapeutic efficacy, considering the safety.

The transfer of the drug through the CRRT compartments is described as

$$k = \frac{Flow\ rate}{V},$$

where k is the transfer rate, V is the compartment volume, and the $flow\ rate$ is defined by $Q_{blood\ flow\ rate}$ or $Q_{effluent\ flow\ rate}$, which has the relation

$$Q_{effluent\ flow\ rate} = Q_{blood\ flow\ rate} + Q_{ultrafiltration\ flow}.$$

Pharmacokinetic parameters inter-individual variability and inter-occasion variability are assumed to be normally distributed. Residual unexplained variability is also considered in the model. If the model is developed purely theoretically or via a theorethical addition, a categorical variable identifying data from a specific study is included to ensure applicability without preferential bias. The applied covariates are divided into baseline continuous covariates – age and body weight, and they are described using the following relation

$$\theta_{individual} = \theta_{population}.\left(\frac{Covariate\ value_{individual}}{median\ Covariate\ value}\right)^{\theta_{covariate}}.e^{\eta_i}$$

and categorial – gender and CRRT modality

$$\theta_{individual} = \theta_{population}.e^{\theta_{covariate}}.e^{\eta_i},$$

where $\theta_{individual}$ and $\theta_{population}$ the individual and the population parameter estimate, $median\ Covariate\ value$ is equal to the median covariate value in the population, $\theta_{covariate}$ is the covariate effect coefficient, and η_i represents the individual random effect. In order to additional covariates to be included, supplementary tests are performed to assess their influence on the model and the necessity of their implementation by estimating a covariate effect coefficient. The applied CRRT modality also contributes to the determination of clearance, for which three main equations are correspondingly defined.

$$Clearance_{Continuous\ Veno-Venous\ Hemofiltration} = \frac{Q_{ultrafiltration\ flow\ rate}.S_c}{1000},$$

$$Clearance_{Continuous\ Veno-Venous\ Hemodialysis} = \frac{Q_{dialysate\ flow\ rate}.S_d}{1000},$$

$$Clearance_{Continuous\ Veno-Venous\ Hemodiafiltration} = \frac{(Q_{ultrafiltration\ flow\ rate} + Q_{dialysate\ flow\ rate}).S_d}{1000},$$

where S_c is the sieving coefficient and S_d is saturation coefficient [5][6][7].

Results

Results from clinical trials demonstrate significant superiority of high-volume HDF in critically ill patients:

- Survival: The ESHOL study showed a 30% reduction in overall mortality and a 33% reduction in cardiovascular mortality with the use of HDF with a convective volume > 23 L/session. The CONVINCE study confirmed these data, reporting a lower risk of death (HR 0.77) with high-dose HDF compared to high-flux hemodialysis.
- Infection control: HDF demonstrated a 55% lower infectious mortality, explained by improved clearance of granulocyte-inhibiting proteins (GIP) and pro-inflammatory cytokines.
- Hemodynamic stability: Convective methods (CRRT/HDF) provide better cardiovascular stability and fewer episodes of intradialytic hypotension (28% reduction in ESHOL). This effect is attributed to more effective plasma refilling and the cooling effect of the replacement fluid, which maintains peripheral vascular resistance.
- Biochemical control: HDF provides significantly higher clearance of β2-microglobulin (reduction up to 80%) and better control of hyperphosphatemia compared to IHD [8][9][10]

Discussion

Hemodynamic challenges

Intradialytic hypotension (IDH) is the most frequent acute complication and is directly associated with end-organ ischemia (myocardial “stunning”, cerebral hypoperfusion) and increased mortality. In sepsis, SA-AKI can occur even at normal systemic pressure due to microcirculatory heterogeneity and interstitial edema, which increases the diffusion distance for oxygen. HDF counteracts IDH through thermal control (energy removal via the replacement fluid), preventing paradoxical vasodilation. Endothelial activation promotes leukocyte and platelet adhesion, increasing the risk of microthrombosis. Severe hypotension often necessitates premature termination of the procedure, leading to under-dialysis. [11] [12]

Coagulation challenges

Patients in septic shock suffer from complex coagulopathy (DIC syndrome) with simultaneous tendency toward bleeding and hypercoagulation. The choice of anticoagulant requires balance: unfractionated heparin is preferred due to its reversibility, but carries a risk of heparin-induced thrombocytopenia (HIT). In HDF, heparin doses are 10–25% higher compared to IHD due to the high hemoconcentrate and transmembrane pressure. Regional citrate anticoagulation is an alternative, but requires heightened attention in hepatic dysfunction, frequently encountered in sepsis.

Pharmacokinetic considerations

In sepsis, the volume of distribution (Vd) of hydrophilic medications increases dramatically due to capillary leak and fluid resuscitation. Hypoalbuminemia increases the free fraction of drugs, but only this fraction is subject to dialytic clearance. Diffusion clearance decreases rapidly for molecules > 500 Da, while the addition of convection in HDF significantly accelerates the elimination of antibiotics such as vancomycin, teicoplanin, and piperacillin. This carries a risk of underdosing (e.g. described seizures due to a drop in valproic acid levels after switching to HDF). Therapeutic drug monitoring (TDM) is critical to avoid therapeutic failure. [13][14]

Conclusion

Hemodiafiltration is currently the most effective dialysis therapy in critically ill patients with sepsis. It combines diffusion and

convection, providing superior clearance of medium-molecular-weight toxins and cytokines. Large-scale studies (ESHOL, CONVINCE) confirm a mortality reduction of 14–30%. The method guarantees better hemodynamic tolerability and reduces chronic micro-inflammation. The guidelines of approved consensus documents serve as a reference point, but the clinical decision to initiate RRT, the choice of anticoagulation, and drug dosing must be strictly individualized according to the patient's needs. Treatment does not simply follow a protocol – it must follow the dynamics of the critical condition with respect to the particularities of each individual patient.

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V. Public Health and Health Management Session

Comparative analysis of the use of National Health Information Systems by pharmacists in Bulgaria and Europe

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Introduction: The integration of the National Health Information System in Bulgaria has radically changed how healthcare functions in our country. This study focused on the changes that have occurred for pharmacists and the pharmacy network with the shift to electronic prescriptions. **The purpose** of this article was to trace the digitalization of healthcare in Bulgaria and across Europe through the National Health Information Systems and to examine the impact of this transformation. **Materials and methods:** The literature review was made by searching for combinations of keywords in the databases of Google Scholar, PubMed and ScienceDirect. **Results and discussion:** The analysis revealed differences in digital philosophy in health information systems in Europe. While the Scandinavian systems were designed around the concept of integrated care, where the pharmacist has full access to the patient's medical history, in the Bulgarian – the access is limited to the level of the specific electronic prescription, which deters the performance of a comprehensive pharmacotherapeutic examination and the identification of drug interactions in real time or potential allergies. **Conclusion:** The comparative analysis showed that the Bulgarian digitalization model still operates in a highly fragmented framework, which contrasts with the highly effective integration in other parts of Europe. Without overcoming this barrier, the concept of pharmaceutical care in Bulgaria will remain formal, and the potential of digital technologies to reduce therapeutic risk will remain unused.
Keywords: *pharmacy, national health information system, digitalization, pharmacies*

Introduction

In just a matter of years, Bulgaria managed to achieve digital transformation in the healthcare sector. Since the 2021 launch of the National Health Information System (NHIS), the country has achieved a significant technical milestone. Currently, with the system work over 7250 pharmacists, and more than 3600 pharmacies are connected into the centralized digital ecosystem. (1) However, a critical divide has surfaced between technical infrastructure and human-centric clinical practice.

According to the currently conducted literature review (2019–2026), real-time decision support is a far more important indicator of the success of digital health than the mere digitalization of records. In Bulgaria, the mandatory adoption of electronic prescriptions for antibiotics and diabetes reached a 97% success rate, successfully reducing fraudulent dispensing. (2) Yet, a comparative analysis revealed a fundamental difference in digital philosophy between the Bulgarian model and other European countries.

While Scandinavian systems in Denmark and Sweden were designed around integrated care of granting pharmacists full access to a patient's medical history, the Bulgarian framework remained concentrated on the document transfer. (3) In the current NHIS system, a pharmacist's access is restricted to the specific prescribed medications in the e-prescription. This limited information hinders the execution of thorough pharmacotherapeutic assessment and the immediate detection of unwanted drug interactions. Furthermore, while state authorities emphasized system efficiency, professional bodies like the Bulgarian Pharmaceutical

Union highlighted a rising administrative burden and a lack of real-time accuracy in stock-tracking systems like the Specialized electronic tracking and analysis system for medicinal products (SESPA). (4) (5)

This article aimed to highlight the differences in digitalization of healthcare across Bulgaria and Europe and to evaluate the impact of this transformation on the pharmacist's role. Hence, without overcoming the current fragmented framework in Bulgaria, the concept of pharmaceutical care becomes formal, leaving the potential of technologies to mitigate therapeutic risk unutilized.

Materials and methods

To evaluate the current state and professional impact of the National health information system (NHIS) in Bulgaria relative to European standards, a multi-dimensional research design was employed that transitions from high-level policy review to a granular analysis of interactions between the pharmacist and the system. The methodology began with a systematic literature review conducted across Google Scholar, PubMed and ScienceDirect databases using combinations of keywords such as "pharmacy", "national health information system", "digitalization", and "pharmacies". The selected filter period for these materials was 2019 to 2026, ensuring that the study captured the trajectory from early digital adoption to the full mandatory integration of electronic records.

Results and discussion

Health systems in Europe used different approaches and Bulgaria focused on documents, not on patients. On the other hand, Scandinavian countries gave pharmacists full access to a patient's medical history. This helped them provide integrated care. In contrast, the Bulgarian system only shows the current e-prescription to the pharmacist. Because of this, medical specialists cannot easily detect harmful drug interactions or allergies. (6)

Furthermore, other European health information systems track medicine shortages. Belgium uses a highly advanced tool. It tracks drug stock in real time and warns pharmacists before shelves are empty. (7) Bulgaria uses a system called SESPA to track drugs. However, the Bulgarian Pharmaceutical Union reports that it lacks real-time accuracy. (8) Furthermore, Nordic countries use automated alerts to stop bad drug combinations instantly. (9) Bulgaria still relies on pharmacists to find these problems manually.

Current research on these systems had major weak spots. Many studies just counted the number of digital prescriptions that were sent. They did not measure whether patient health actually improved. Researchers also focused too much on screen clicks instead of watching how technology changed real interactions with patients. Finally, most studies only looked at regular community pharmacies, leaving hospital pharmacies highly under-researched. (10) (11)

Research indicated that Nordic and Baltic countries (and recently Bulgaria) have high-functioning integrated systems, while larger economies like Germany and France are still navigating the complexities of transitioning from legacy monolithic architectures to modular, cloud-native platforms. (12) Despite these technical successes, the results indicate a profound separation in the key components between the Bulgarian model and leading European systems.

The analysis revealed distinctions in health information systems in Europe, shown in Table 1.

Table 1. Comparison of health information systems in Europe

Access type	**High access (Estonia, Finland, Denmark)**	**Moderate access (Bulgaria, Poland, Spain)**	**Restricted access (Germany, Austria, France)**
Complete drug history	Yes. Available for up to 5+ years to prevent harmful interactions.	It usually only sees active recipes.	Limited. Often they only see the specific prescription.
Laboratory results	Yes. They may check kidney/hepatic function for dose adjustment.	No. It is usually limited to doctors only.	No. The patient must provide a paper or digital copy manually.
Digital dosing	Automated.	In real time. Linked to a national identifier or QR codes.	Hybrid. They are switching to QR codes; paper archives are common.
Generic substitution	Required. The system imposes the lowest price.	Allowed. It is allowed if the doctor has not "locked" the medication.	Strict rules. Germany limits flexibility in substitutions.
Allergy alarms	Automatic. The system marks contraindications from the electronic health record (HER).	Manual. It depends on the local pharmacy software.	Manually. The pharmacist must ask the patient.

Estonia, Finland, and Denmark feature highly integrated digital health systems that automate generic substitutions, allergy alarms and dosing, in addition to providing pharmacists with access to patients' medication histories and laboratory results. Moderate-access nations such as Bulgaria, Poland and Spain rely on partially connected systems that offer real-time digital dosing and flexible generic substitutions but restrict pharmacists to viewing only active prescriptions without access to clinical lab data. Germany, Austria, and France have fragmented, hybrid systems where pharmacists have limited prescription visibility and must rely heavily on communication with the patient to check for allergies or review laboratory results.

As of 2026, only specific countries were fully integrated into the MyHealth@EU system, which allows pharmacists in one country to retrieve and dispense digital prescriptions from another. (13) Bulgaria is currently working on the integration with the cross-border e-prescriptions, which will allow the ability for an EU patient to obtain medication in Bulgaria and vice versa. Also, other forthcoming projects are an interactive map of pharmacies for better access to pharmaceutical care and new versions of the DRUMS (System for the registration of medicinal products for the purposes of the Executive Agency for Medicines) and SESPA systems. (1)

The digitalization of the sector promises to revolutionize patient healthcare globally. From different technologies, virtual tools, including artificial intelligence, blockchain, virtual and augmented reality, provide significant benefits to patients and the pharmaceutical sector alike, ranging from improving access to clinicians and medicines, as well as improving real-time diagnoses and treatments. Indeed, it is envisioned that such technologies will communicate together in real-time, as well as with their physical counterparts, to create a large-scale, cyber healthcare system. Despite the significant benefits that virtual-based digital health technologies can bring to patient care, a number of challenges still remain, ranging from data security to acceptance within the healthcare sector. The Pharmacy 4.0 concept represents the next stage in the evolution of pharmaceutical practice. The key features of this model include cyber-physical healthcare systems where virtual tools and physical components communicate with each other to

create a large-scale, integrated patient care network. Additionally, automation and clinical support where the shift is moving from simple administration to intelligent systems that provide alerts for drug interactions and automated dosing. Furthermore, Pharmacy 4.0 enables pharmacists to use large datasets for preventive diagnostics and real-time monitoring of therapeutic outcomes. (14)

Conclusion

The evolution of the Bulgarian National Health Information System represented one of the most rapid digital transformations in the Balkan region. While it has successfully transitioned from a paper-based bureaucracy to a centralized electronic prescription model, the system currently functions primarily as a transactional tool rather than a clinical one.

In comparison to Europe, the Bulgarian pharmacist remained digitally isolated from the broader clinical picture. To fulfil the objectives of the European Health Data Space (EHDS), Bulgaria must shift its focus from data collection to data utilization. (15) By empowering pharmacists with deeper clinical insights and cross-border capabilities, Bulgaria can significantly improve patient safety and public health outcomes.

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Prevention and rehabilitation of calf muscle ruptures in professional football players with a history of achilles tendinopathy

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Introduction: Achilles tendinopathy is a prevalent degenerative overuse injury among professional football players and constitutes a significant endogenous risk factor for secondary muscle ruptures in the sural region. Chronic alterations within the tendon impair the elastic, biomechanical, and neuromuscular characteristics of the triceps surae muscle-tendon unit, thereby increasing the risk of injury during high-velocity and explosive sporting maneuvers. **Aim:** The objective of this report is to analyze the correlation between Achilles tendinopathy and calf muscle ruptures in professional football players, and to define effective strategies for primary and secondary prevention and rehabilitation. **Materials and methods:** A systematic analytical review of contemporary scientific literature was conducted, complemented by a clinical case study involving a professional football player with a history of Achilles tendinopathy and a subsequent rupture of the m. gastrocnemius. Functional and clinical assessments were utilized, including strength testing, range of motion (ROM) mobility tests, postural balance, and return-to-sport readiness protocols, alongside a multimodal rehabilitation program. **Results and discussion:** Key risk factors were identified, including restricted ankle dorsiflexion, muscle asymmetries, impaired motor control, and inadequate load management. The implementation of an integrated rehabilitation approach-incorporating physiotherapy modalities, eccentric and heavy-load resistance training of the calf musculature, plyometrics, and sport-specific drills-resulted in a shortened recovery period and a significant reduction in the rate of recurrence. **Conclusion:** Achilles tendinopathy should be addressed as a systemic dysfunction of the muscle-tendon balance. Early diagnosis, individualized prevention programs, and comprehensive rehabilitation are essential for optimizing athletic recovery and ensuring long-term career longevity in professional football.
Keywords: *achilles tendinopathy, rehabilitation, sports injury prevention*

Introduction

Achilles tendinopathy is an overuse condition characterized by impaired collagen organization, decreased mechanical efficiency, and alterations in neuromuscular control. In professional football, cyclic-explosive loads – such as sprinting, acceleration, changes of direction, and jumping-increase peak tension within the triceps surae-Achilles complex. This elevates the risk of secondary calf muscle injuries, particularly when there is insufficient tissue capacity or an imbalance between acute and chronic workloads [1, 2, 3].

Biomechanically, the Achilles tendon functions as an energy-storing element. During dorsiflexion, elastic energy is accumulated, which is subsequently released during the push-off phase. In cases of tendinopathy, these elastic properties deteriorate, leading to increased ground contact time and a greater dependence on the active work of the m. gastrocnemius and m. soleus. This can result in compensatory gastrocnemius dominance during explosive movements and heightened eccentric stress within the calf [4, 5, 6].

Epidemiologically, muscle injuries (including those of the calf) are a leading cause of absence in professional football, carrying a high risk of recurrence if there is inadequate load progression or a mismatch between tissue capacity and exposure to high-velocity actions. Eccentric protocols and heavy slow resistance (HSR) training have proven effective in treating Achilles tendinopathy by increasing tissue tolerance and functional capacity [7, 8, 9]. Furthermore, the FIFA 11+ program has demonstrated success in reducing injury rates by improving strength, balance, and neuromuscular control [10, 11].

Objective

To present a clinical case study (n = 1) and a structured 8-week protocol for the assessment, rehabilitation, and secondary prevention of calf muscle injuries in a professional football player (29 years old, center-forward, right-leg dominant) with chronic Achilles tendinopathy (2-month duration). The study monitors validated functional indicators and established return-to-play (RTP) criteria.

Material and methods

The subject of this case study (n = 1) is a 29-year-old professional football player (striker), right-limb dominant. Chronic symptoms with a duration of two months. Primary complaints include pain and stiffness in the mid-portion of the Achilles tendon during initial movement "first steps" and during explosive actions. Additionally, the participant reports tension and pain in the medial aspect of the calf during maximal-velocity running. Inclusion Criteria: clinical presentation of Achilles tendinopathy (load-related pain, morning stiffness, and localized tenderness along the tendon); pain provoked during high-velocity movements, with no clinical evidence of acute total rupture; ability to perform submaximal exercises with an acute pain intensity of ≤ 5/10 on the Visual Analog Scale (VAS).

To evaluate therapeutic progression and determine Return-to-Play (RTP) readiness, the following validated tools are utilized: Pain Assessment (VAS, 0–10): Quantitative assessment of pain during daily activities and sport-specific loading (sprinting/acceleration); VISA-A Questionnaire (0–100): A validated, Achilles-specific tool sensitive to changes in symptom severity and functional capacity; Single-Leg Heel-Rise Endurance Test: Measured by the number of repetitions to failure using a standardized technique; serves as an indicator of plantar flexor endurance and function; Weight-Bearing Lunge Test (WBLT/Knee-to-Wall, cm): An indirect measure of ankle dorsiflexion range of motion in a weight-bearing position; associated with injury risk and lower-limb kinematics; Reactive Strength Index (RSI): Derived from a Drop Jump; serves as a marker for Stretch-Shortening Cycle (SSC) efficiency and tendon reactive strength; 20 m Sprint Test (sec): A functional performance indicator used to assess the return of explosive speed and tolerance to maximal acceleration; The Limb Symmetry Index (LSI) was calculated for both the heel-rise endurance and the RSI, with a threshold of ≥ 90% established as the criterion for RTP [9].

Therapeutic intervention: 8-week combined protocol

The protocol is based on a multidisciplinary approach, integrating isometric loading, Heavy Slow Resistance (HSR), eccentric remodeling, plyometrics, and a specific progression toward maximal speed. The primary therapeutic principle is the systematic increase of tissue capacity and mechanical tolerance of the Achilles tendon prior to exposure to high-velocity peak loads. The prophylactic framework is supplemented by the FIFA 11+ program for secondary prevention.

Phase 1: Load control and analgesia (Weeks 1–2)

- Isometric Plantarflexions: Performed with both straight and flexed knee (targeting the *m. gastrocnemius* and *m. soleus*). Dosage: 4–5 sets × 45 sec, 1–2 times daily.
- Ankle Mobility: "Knee-to-wall" progressions. Dosage: 3–5 sets × 8–10 repetitions, daily.
- Core Foundation: Exercises for anti-extension and anti-rotation (e.g., Pallof press, Plank variations). Dosage: 2–3 sessions per week.

Phase 2: Strength loading and tissue remodeling (Weeks 3–4)

- HSR Calf Raises: High-resistance, low-velocity heel raises. Dosage: 3–4 sets × 6–8 repetitions with progressive loading, 3 times per week.
- Eccentric Heel Drops: Modified Alfredson protocol according to individual pain tolerance. Dosage: 3 sets × 15 repetitions, 2 times daily.
- Soleus-dominant Training: Seated calf raises. Dosage: 3–4 sets × 10–12 repetitions.
- Neuromuscular Control: Single-leg balance and dynamic stability (Y-balance exercises) with a focus on knee and pelvic alignment.

Phase 3: Reactive strength and plyometric progression (Weeks 5–6)

- Low-level Plyometrics: Drop landing technique and light hops. Emphasis on stabilization time and control. Dosage: 2 times per week.
- Single-leg Hops: For dynamic stability and reactivity. Dosage: 3 sets × 6–8 repetitions.
- Strength Maintenance: Continuation of the HSR protocol 2 times per week.

Phase 4: Sport-specific integration and RTP (Weeks 7–8)

- Repeated Sprints: 6-10 repetitions at 90–95% of maximal speed.
- Change of Direction (COD): Progressive increase in cutting angles (45°–90°–180°).
- Small-Sided Games (SSG): Integration into team training via small-scale games involving rapid acceleration and deceleration.

To ensure secondary prevention, the FIFA 11+ protocol was implemented 2–3 times weekly [10, 11].

Results

The quantitative changes in the assessed functional parameters following the 8-week therapeutic protocol are summarized in Table 1.

Table 1. Dynamics of functional parameters (Week 1 vs. Week 8)

Parameter	Week 1	Week 8	Change (%)
VISA-A (Score)	54	89	+64.8%
VAS (Pain)	6	0–1	−83% to −100%
Heed-rise (Reps)	19	31	+63.2%
Knee-to-wall (cm)	8 cm	12 cm	+50%
RSI (Index)	1.15	1.72	+49.6%
20 m Sprint (sec)	3.12 s	2.97 s	−4.8%

The VISA-A scores demonstrate a transition from moderate functional limitation to a level compatible with a full Return-to-Play (RTP). A clinically significant reduction in pain was observed via the VAS, with no signs of reactive inflammation or post-loading exacerbation. Muscle endurance, measured by the Heel-rise test, improved substantially, with the Limb Symmetry Index (LSI) reaching 97%. The normalization of weight-bearing dorsiflexion (+50%) and the 49.6% increase in the Reactive Strength Index (RSI) confirm the effective restoration of the stretch-shortening cycle (SSC). The improvement in the 20 m sprint (−4.8%) carries high functional value given the athlete's specific playing position as a forward.

Discussion

These results support the thesis that progressive mechanical loading is the primary therapeutic mechanism for managing Achilles tendinopathy [7, 8, 12]. The restoration of Range of Motion (ROM) and the enhancement of soleus-dominant capacity likely reduce eccentric stress on the gastrocnemius, thereby protecting the calf complex from secondary injuries. The positive dynamics in RSI serve as an indicator of recovered

reactive strength-a critical factor for efficiency during acceleration and Change of Direction (COD) [13, 14].

The decision for Return-to-Play (RTP) in professional athletes must be based on a multifactorial analysis, including: Minimal subjective pain; LSI ≥ 90% across functional tests; Tolerance to loading at speeds exceeding 90% of maximal capacity; Restored dorsiflexion volume [1, 3, 9, 15].

The integration of the FIFA 11+ program proved to be a scientifically sound strategy for systematic secondary prevention [10, 11, 16]. The primary limitation of this study is its nature as a single case report (*n = 1*).

Conclusion

The implementation of a combined 8-week protocol for a professional football player with Achilles tendinopathy resulted in a comprehensive therapeutic effect:

- Significant functional optimization (+64.8%) and total pain reduction;
- Restoration of strength-endurance capacity (+63.2%) and dorsiflexion (+50%);
- Improvement in reactive strength (+49.6%) and sprint velocity.

The FIFA 11+ program should be considered a mandatory, permanent component of the secondary prevention strategy following the rehabilitation phase.

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Knowledge and attitudes regarding preconception care among women of reproductive age: differences according to age and educational attainment – a narrative review

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Preconception care (PCC) represents an essential component of reproductive health and preventive medicine, aimed at reducing adverse maternal and perinatal outcomes. The effectiveness of this approach is determined by the level of awareness and the attitudes of women of reproductive age. The aim of the present narrative review is to analyze the available data on the knowledge, attitudes, and perceptions of PCC, with an emphasis on age- and education-related differences. The results show that a higher educational attainment and increasing age are associated with significantly better awareness, more favorable attitudes, and a higher likelihood of adopting recommended health behaviors. Women with higher educational attainment more often seek professional medical consultation and use digital health resources, whereas those with lower education more often rely on informal sources of information and underestimate the importance of preventive measures. Younger and nulliparous women, as well as women with fewer years of formal education, tend to demonstrate poorer awareness, weaker motivation, and lower uptake of PCC services, as well as a higher frequency of misconceptions regarding the scope and necessity of care. Access to reliable and structured information, social support, and digital literacy play a key role in shaping adequate health beliefs and behaviors during the preconception period. Improving the effectiveness of PCC requires the implementation of targeted interventions, integration of digital tools, and active participation of healthcare professionals in the educational process.

Keywords: *preconception care, reproductive health, educational level, maternal age, perinatal outcomes, health attitudes*

Introduction

Preconception care (PCC) is established as a key component of reproductive health and preventive medicine, with proven potential to reduce adverse maternal and perinatal outcomes [1–4]. The World Health Organization (WHO) defines this approach as a set of medical, behavioral, and social interventions applied during the period before conception, aimed at optimizing the health status of the woman and the couple [5, 6]. The scope of PCC includes family planning, modification of risk behaviors (smoking, alcohol use), management of chronic diseases, immunizations, genetic counseling, and promotion of a healthy lifestyle [6].

The importance of this approach is underscored by the high proportion of unplanned pregnancies (approximately 45%) and teenage pregnancies (approximately 12%), which are associated with an increased risk of adverse neonatal outcomes [4]. A critical aspect is the fact that organogenesis occurs between days 17 and 56 after conception – a time window during which a considerable proportion of women have not yet recognized their pregnancy. This limits the effectiveness of interventions initiated after pregnancy diagnosis and emphasizes the necessity of early prevention [6].

In this context, PCC is regarded as an essential element of the concept of "continuity of care", which ensures a coordinated and integrated approach to health services across the different stages of the life cycle [7, 8].

Despite the existence of established recommendations, the utilization of PCC remains limited. One of the main determinants is the level of health literacy, which varies considerably depending on age, education, and socioeconomic status.

The aim of this review is to analyze the knowledge, attitudes, and perceptions of PCC among women of reproductive age, with an emphasis on the influence of age and educational levels.

Materials and methods

The present study is a narrative review based on a systematic search of PubMed, Google Scholar, and Web of Science databases.

The search was conducted using a combination of keywords and MeSH terms, including: “preconception care”, “preconception health”, “knowledge”, “attitudes”, “reproductive age”, “health literacy”, as well as their Bulgarian-language equivalents. Boolean operators (AND, OR) were used to optimize the results.

The analysis included original research publications and review articles published between 2007 and 2025. Selection of sources was conducted through a two-stage process, comprising an initial screening by title and abstract, followed by an assessment of full texts for relevance to the study objectives.

Inclusion criteria covered publications examining knowledge, attitudes, and practices related to PCC among women of reproductive age. Studies with limited access to the full text, publications outside the defined time range, and studies focused exclusively on specific clinical groups without broader population-level relevance were excluded.

A descriptive analytical approach was used to systematize the data, whereby the results were grouped into thematic categories, including awareness, health attitudes, behavioral patterns, barriers to service use, and factors influencing health behavior.

Where applicable, an assessment of the methodological quality of the included studies was performed based on design, sample size, and potential sources of bias, without applying a formal standardized quality assessment tool.

In interpreting the results, limitations related to the heterogeneity of the studies included and potential publication bias were considered.

Results

Despite the existence of established recommendations, PCC remains fragmented and underutilized. The main barriers include low awareness among the population and socioeconomic constraints such as low educational level, financial difficulties and lack of support, unclear distribution of responsibilities among healthcare professionals, as well as restricted access to services [9–11].

Risk behaviors remain widespread, with 33% of women smoking, 64.4% consuming alcohol, and 9.6% using psychoactive substances [12]. Although healthy eating and physical activity are recognized as important, awareness of specific PCC risk factors remains limited, particularly among nulliparous and younger women [13].

Education and age are among the most consistent determinants of PCC-related knowledge, attitudes, and preventive behaviors (Table 1). A lower educational attainment is associated with poorer health literacy, more poor awareness, and a lower frequency of adopting recommended health practices, whereas a higher educational attainment is associated with better knowledge and more frequent uptake of recommended health behaviors [12, 14–17].

Age also has a considerable influence, with older women demonstrating a more positive attitude toward PCC and greater engagement in preventive practices, whereas younger women show lower levels of awareness and engagement [12–14, 18, 19].

Socioeconomic status influences access to information and health services, with women in more disadvantaged social positions more often demonstrating lower awareness and lower uptake of PCC services [20–23].

Digital sources of information are perceived as widely acceptable, but differences are observed in their use depending on educational level, with more highly educated

women more often using them actively and effectively [13].

Social support, including partners and family, is considered important because it facilitates the uptake of recommended health behaviors and increases engagement with PCC [12, 13, 17, 22, 23].

There are substantial differences in attitudes, concerns, and perceptions of PCC between nulliparous women and pregnant or parous women, with reproductive experience emerging as a significant factor (Table 2). Women with prior reproductive experience demonstrate higher health literacy and a better understanding of risks, but at the same time show higher anxiety, particularly in the context of complications or adverse outcomes [13, 24–27].

Table 1. Influence of education and age on knowledge, attitudes, and behavior related to PCC

Criterion	**Lower educational attainment / younger age**	**Higher educational attainment / older age**
Knowledge and awareness	Limited knowledge, low health literacy	Higher awareness; better knowledge (AOR = 28.55)
Behavior	Lower frequency of adherence to recommendations	Higher frequency of healthy practices (AOR = 2.49)
Perceived importance of PCC	Low priority	Higher significance; more positive attitude at age >35 years (AOR = 8.73)
Healthy behavior (age)	Less frequent adoption	Higher likelihood at age > 35 years (AOR = 3.16)
Knowledge of risk factors	Limited (folic acid, BMI, birth intervals)	Knowledge of specific risk factors remains incomplete, particularly regarding folic acid use, optimal weight, age-related risks, and birth spacing. Even among women with better general awareness, understanding of some PCC components remains limited
Digital sources	Limited and less effective use	Active use; 99.5% acceptability
Access to services	Limited access	Better accessibility; among low-income groups – 3.11-fold lower awareness

Table 2. Influence of reproductive experience on knowledge, attitudes, and behavior

Criterion	**Nulliparous women**	**Parous / pregnant women**
Overall awareness	Lower level of awareness and limited scope of knowledge	Higher level of awareness, associated with prior experience
Knowledge of risk factors	Limited, mainly theoretical	Better, practically oriented
Main concerns	Uncertainty and information deficit	Concerns regarding complications and outcome
Anxiety	Lower (possible underestimation of risk)	Increased (especially in high-risk pregnancy)
Attitude toward medical interventions	Hesitancy and uncertainty	Concerns about excessive medical intervention
Sources of information	Social networks and informal sources	Medical professionals and reliable sources

Nulliparous women show lower awareness and more limited knowledge of risk factors, with their knowledge being predominantly theoretical. They more often exhibit uncertainty and an information deficit, as well as a lower degree of perceived risk. Differences are also observed regarding sources of information, with nulliparous women more often relying on informal channels, whereas women with reproductive experience predominantly rely on medical professionals as their main source of information [13, 25–27].

In this context, contemporary PCC educational programs are regarded as a key tool for overcoming disparities in awareness and for supporting women at different stages of reproductive life. The data show that these interventions are associated with improvements in knowledge, attitudes, and health behaviors, as well as favorable effects on mental health and biometric indicators [28, 29].

In-person training remains the most used model, with group sessions facilitating social support and the exchange of experience, while individual counseling allows for a personalized approach and targeting of specific risk factors. Printed materials are used as a supplementary resource but have limited effectiveness, particularly among women with low health literacy [28].

Technology-based interventions, including websites, chatbots, and electronic communication, provide continuous access to information and opportunities for personalized risk assessment. The highest effectiveness is observed when digital tools are combined with direct interaction with a healthcare professional [28, 29].

Among the interventions applied, programs aimed at changing health behavior and increasing awareness stand out, as well as models based on integration into primary health care [30, 31]. Additionally, interventions targeting healthcare professionals and the community show positive results regarding health behaviors and adherence to recommendations [32–34].

The available data supports the implementation of an integrated, life-course-based PCC model that includes educational, digital, and community components, with the potential to improve health outcomes and reduce social inequalities [29].

Discussion

Health literacy is central to influencing maternal and neonatal outcomes. Data from a study among 10,038 nulliparous women show that 19% of pregnant women have inadequate health literacy, which is associated with an increased risk of adverse outcomes, including cesarean section, severe perineal lacerations, small-for-gestational-age birth, low birth weight, and a low 5-minute Apgar score [34].

The influence of health literacy operates through several key mechanisms, including access to and effective use of health services, the quality of patient–provider communication, and the ability to establish and maintain healthy behavior. These factors are of particular importance during the period before and during pregnancy, when early identification and modification of risk factors are essential for pregnancy outcomes [34, 35].

Low health literacy is associated with a limited understanding of key reproductive topics, including contraception, prenatal genetic screening, gestational diabetes, and preeclampsia, as well as with a lower frequency of seeking PCC [29, 34]. These observations are consistent with the findings of the present review, which show lower awareness and more limited use of preventive services among women with fewer years of formal education and a more disadvantaged socioeconomic status.

In this context, effective interventions should be directed toward improving health literacy through the integration of counseling into primary health care, training of healthcare professionals, and the implementation of multicomponent approaches involving supplementation, digital resources, and personalized support [1, 29, 34]. Combining different intervention strategies allows both information deficits and behavioral and structural barriers to be addressed.

Conclusion

PCC represents an essential component of preventive medicine that provides an opportunity for the early identification and modification of risk factors to improve maternal and perinatal outcomes. The main determinants of its use include educational level, age, and socioeconomic status, with the most vulnerable groups remaining those with limited access to such services.

Systematic integration of PCC into primary health care is needed, along with an expansion of interventions aimed at improving health literacy through educational and digital approaches. Targeted programs directed at young women and at-risk populations are of particular importance.

An integrated, multidisciplinary, and accessible PCC model may improve reproductive health, reduce inequalities, and contribute to better short- and long-term outcomes for mothers and children.

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Conducting a pilot study on leading stressors and stress control capacity in first-year students using the biofeedback method

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Introduction: Stress is an inherent aspect of human life that cannot be eliminated but rather managed. Young individuals transitioning to university are subjected to a multitude of stressors due to inevitable changes in lifestyle, academic demands, and the formation of new relationships. Consequently, developing effective stress management strategies is paramount for preserving mental and physical well-being. This study aims to investigate the capacity of young adults to recover from various types of stressors using the Biofeedback methodology. **Materials and methods:** To achieve the study's objective, the Mind Reflection Biofeedback system was employed. Ten cadets, aged 19 to 21, participated in the study. They were exposed to different stressors, including cognitive load, time-sensitive decision-making, and emotional burden. The participants were situated in a relatively controlled environment throughout the experimental procedure. **Results and discussion:** The findings indicate that four participants demonstrated the ability to recover from stress. One individual exhibited partially developed these capacities. Visualization of the results through the hardware methodology allowed for the generation of individual stress profiles. This facilitated the determination of the impact of each stressor and the participants' recovery capabilities, providing a clear picture of their current potential and areas for future development. **Conclusion:** The obtained results serve as a foundation for further investigation. Increasing the sample size will either validate or refute the observations made this far.

Keywords: *Stress, Stressor, Biofeedback, Stress Management*

Introduction

Stress is often defined as the "disease of the 21st century" due to its widespread presence and significant impact on the mental and physical health of modern individuals. One of the life crises experienced by young people is the transition to higher education. Entering university is associated with the transition to early adulthood and represents a silent life crisis connected with separation from the family environment, adaptation to a new lifestyle, personal responsibility, and identity formation. This transition is accompanied by increased levels of stress, anxiety, and uncertainty. Successful adaptation leads to the development of new social and personal skills, while unsuccessful adaptation may result in discontinuation of education. The question remains to what extent young people are prepared to cope with these new challenges and whether they possess developed stress management skills.

Exposition

Theoritical framework

According to Erik Erikson's psychosocial theory of development, the period of early adulthood is characterized by the crisis of "intimacy versus isolation", preceded by the crisis of "identity versus role confusion" in adolescence (Erikson, 1963, 1968). Entering college often coincides with these important developmental stages, during which a young person constructs his or her identity, value system, and life goals. Failure to resolve these internal conflicts can increase experiences of stress and uncertainty in the new academic environment. The development of coping mechanisms depends on the individual and their perception of the environment. The model by Richard Lazarus and Folkman views stress as the result of a two-way interaction between the individual and the environment. According to them, stress is not viewed solely as an external impact or

physiological reaction, but as the result of the interaction between the individual and the environment, in which the cognitive appraisal of the situation plays a key role (Lazarus & Folkman, 1984). They conclude that an event is experienced as stressful only when the individual evaluates it as threatening or exceeding his or her available coping resources. In this context, individual differences, personality characteristics, and available coping strategies are determining factors in the consequences of stress.

Young people entering higher education are particularly vulnerable to stress. The period of adaptation to the university environment is accompanied by numerous changes, including increased academic demands, the need for independent time planning, financial responsibility, and the development of new social relationships. These factors are often assessed as significant stressors and can cause increased levels of anxiety, mental tension, and emotional instability.

In this sense, building effective models for coping with stress is essential for maintaining the mental and physical health of students. As Lazarus emphasizes, successful coping with stress depends not so much on the event itself, but on the way it is interpreted and the available resources for adaptation. Social support and self-regulation skills are key to increasing resilience to stress and for more successful integration into the academic and social environment.

Aim

The aim of this study is to examine the ability of young people to recover after exposure to different types of stressors using the biofeedback methodology.

Materials and methods

A device technique is used – Mind Reflection GRS, measuring the impact of various stress factors on the psyche, using the biofeedback method through galvanic skin response.

The biofeedback method is a non-invasive procedure that connects the mind and body, and helps to strengthen physical and mental well-being. In this study, the Mind Reflection GRS device technique is used. It allows you to visualize bodily signals and manifestations of tension and anxiety (such as rapid pulse, increased temperature and muscle tension, etc.). This makes it possible to analyze the relationship between external stimuli and the body's reactions to stress and, accordingly, to improve the personal physiological way of dealing with it. The Mind Reflection GRS device measures the electrical activity of the skin – changes in sweating – in order to register the reactions of the examined person to sudden emotional stress caused by various stress factors and the individual's efforts to cope with them. Changes in the electrical resistance of the skin are recorded by a sensor that is placed on the index and middle fingers of the examined person. The frequency of measurement of changes in the electrical resistance of the skin is 10 Hz (10 measurements per second).

Research methodology

1. Informing the volunteers in the study.

2. Filling out declarations of consent in accordance with the Ethics of scientific research

3. Conducting the study in a relatively controlled environment – classroom, temperature 22–23 degrees, normal lighting

Students studied: 10 students aged between 19 and 21 years.

The research methodology is based on biofeedback. It provides objective results through the use of galvanic skin response, which takes into account the impact of a certain stressor on the personality. The study

involves recording the personality's reactions to the impact of various stress factors – cognitive, emotional and mental. By measuring changes in the electrical conductivity of the skin, the physiological changes that occur during the impact of stress are determined. These changes are detected through galvanic skin response. Typically, changes in the electrical resistance of the skin occur no later than 2 to 3 seconds after the appearance of a new or significant stimulus for the subject (Stavrev, D., 2022). Electrical skin resistance is an indicator that is sensitive to the impact of opposing states such as internal resistance or acceptance of the stressor; emotional response with anxiety or calmness; excessive volitional effort and the ability to relax; inclusion or detachment from an external stimulus provided by the researcher or an internal stimulus provided by the subject himself. This allows us to track the influence of different stimuli on the way we respond to stress and the ability to recover during breaks between them.

Procedure for conducting the study:

First, the subjects are introduced to the purpose and method of conducting the study. They sign an informed consent. The study requires two laptops/computers, a device and sensors. The device is connected to a computer with the appropriate software. The computer processes and analyzes the data it receives from the sensors placed on the index and middle fingers of the leading hand. The software visualizes the data in real time. The second laptop is used to visualize the Stroop test. Then, the subject sits in a comfortable chair and the sensors that measure the galvanic skin response are placed. Different stressors are applied sequentially:

Stressor 1: cognitive load. The volunteer makes calculations out loud on a task given by the experimenter – 2 min.

Recovery break – 2 min.

- Stressor 2 – Stroop test aimed at measuring the ability to make decisions under time constraints – 2 min.

Recovery break – 2 min.

- Stressor 3 – sharing an emotional experience with negative emotions.

Recovery break – 2 min.

With the last stressor, the study ends, and the results are recorded. Then, feedback is given and any questions that arise are discussed.

Results

For 60% of participants the leading stressor was emotional, while for 40% it was decision-making under time pressure. The indicators for the individual stressors are presented in Table 1, “Presentation of the Individual Ranking of Stressors among the Participants.” Under number 1, the stressor with the highest degree of impact on the examined person is indicated; under number 2, the stressor with the next highest degree of impact; and under number 3, the stress factor with the weakest influence on the individual is presented.

Table 1. Individual ranking of stressors among participants

Participant	***Cognitive***	***Decision-Making***	***Emotional***
P1	3	1	2
P2	3	2	1
P3	3	1	2
P4	3	2	1
P5	3	2	1
P6	3	2	1
P7	3	2	1
P8	3	2	1
P9	3	1	2
P10	3	1	2

From the indicators included in Table 1, the following conclusion can be drawn: the

cognitive stressor does not appear as a leading one for any of the participants. This may be explained by the well-developed cognitive abilities of the participants in the study, for whom cognitive load is not a source of stress.

The percentage distribution of the obtained results (Fig. 1) indicates a certain tendency in which the leading stressor is the emotional one, followed by the stressor "decision-making under time deficit." The obtained results may be explained by the lack of experience of the examined persons in managing their time and reprioritizing tasks. Emotional intelligence, especially in the aspect of emotion regulation, is still not well developed. This has a strong impact on the perception of information and explains the significance of the emotional stressor.

In Figure 2, the obtained results show that 40 percent of the examined individuals possess the ability to recover from stress; in 10 percent of them, a partially developed ability is observed, while in the remaining participants this ability is not developed. This may be explained by the fact that at this age psychological mechanisms for coping with tension are still being formed (Stoyanov, V., 2005).

In their daily lives, the examined individuals are subjected to high levels of workload, but they do not have the necessary social experience and coping mechanisms to deal with the increased demands of the environment.

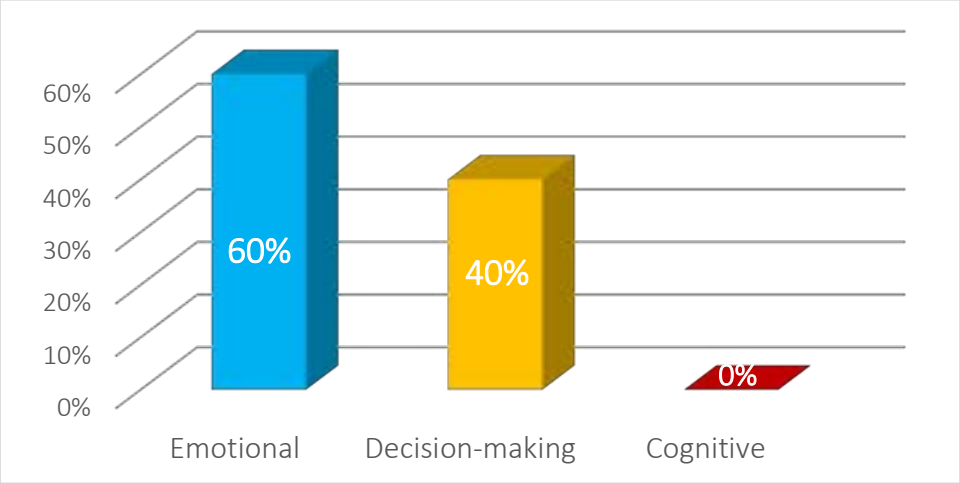

Figure 1. Percentage distribution of the leading stressors among the surveyed individuals

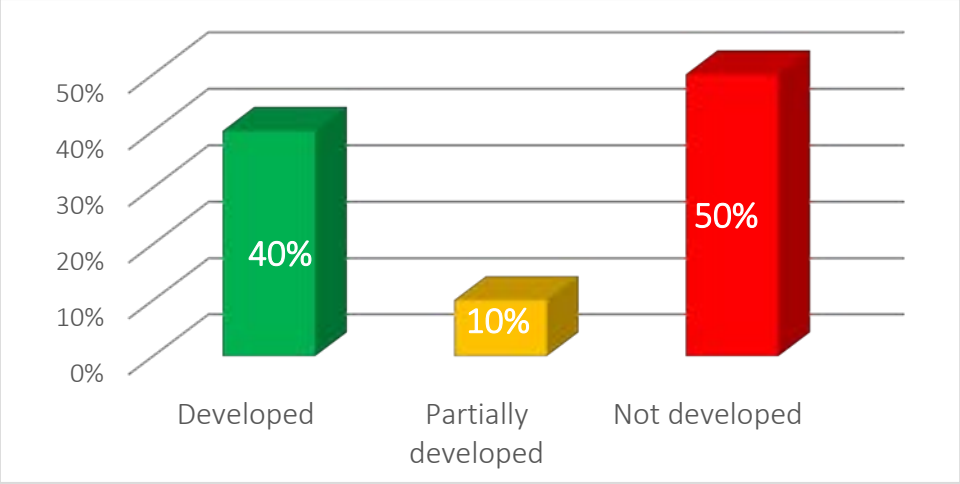

Figure 2. Stress control skills of the surveyed individuals in percentages

Discussion

The benefits of the conducted study can be observed in several directions:

- On an individual level – the individual receives effective and visual feedback regarding their resources for coping with stress, as well as guidance for the development of coping strategies;

- The summarized results provide information about the emotional development and resilience of newly enrolled students and may serve as a basis for the development of various training programs or support systems.

Conclusion

Visualization of the obtained results through the instrumental methodology allows the creation of a stress profile for each person studied. This makes it possible to determine the strength of impact of each stressor and the recovery abilities, giving a picture of the real possibilities and directions for future development. The obtained results serve as a basis for continuing the study. Increasing the number of persons studied will confirm or reject the observations made so far. According to the accumulated data, the influence of emotions on the level of stress remains leading for the personality, as well as the mass accumulation of stress, conditioned by the limited possibilities for effective self-regulation.

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Comparative effectiveness of standard kinesitherapy and neurodynamic exercises in the prevention of chronic vertebrogenic musculoskeletal disorders in office workers

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Introduction: Chronic musculoskeletal disorders of the spinal column are highly prevalent among sedentary office workers, primarily due to prolonged static postures and ergonomic deficiencies. Targeted preventive kinesitherapeutic strategies are essential to mitigate these occupational health risks. **Aim:** To conduct a comparative analysis of the effectiveness of a standard kinesitherapy (SK) program versus a neurodynamic (ND) exercise protocol in the prevention of chronic spinal disorders among office employees. **Materials and methods:** Sixty participants with no significant spinal pathology were randomized into two intervention groups (SK and ND) for a 12-week period. The SK group followed a conventional exercise regimen focused on postural correction, core stabilization, and flexibility enhancement. The ND group performed specialized nerve mobilization techniques targeting major peripheral neural pathways to maintain neural mobility. Outcome measures included the incidence of self-reported spinal pain, flexibility assessments, and neural tension tolerance tests. **Results and discussion:** Both interventions yielded statistically significant reductions in reported pain levels and improvements in spinal flexibility compared to baseline. The ND group demonstrated superior outcomes in neural tension testing and slightly lower pain scores relative to the SK group. Preserving neural mobility through ND exercises appears to provide a synergistic preventive effect by reducing neural strain and hypersensitivity. Concurrently, standard kinesitherapy proved effective in enhancing muscular support and postural stability, confirming that structured physical activity is fundamental for spinal health. **Conclusion:** Neurodynamic techniques may offer distinct therapeutic advantages by maintaining neural health and reducing mechanical stress, potentially enhancing the long-term functional integrity of the spine in sedentary populations.

Keywords: *office workers, neurodynamics, kinesitherapy, prevention*

Introduction

Sedentary work, defined by sustained static postures and restricted movement variability, is linked to a heightened risk of musculoskeletal pain and the development of chronicity, particularly within office-based populat [1]. Research indicates that workplace interventions incorporating structured exercise lead to a significant reduction in neck and shoulder pain and improved functionality among office employees [2, 3], with exercise dosage and adherence serving as critical moderators of the therapeutic effect [4]. Furthermore, sedentary behavior across various domains-both occupational and non-occupational-demonstrates a positive correlation with a higher prevalence of musculoskeletal complaints [1]. Neurodynamics or neural tissue management conceptualizes the nervous tissue as a biomechanically active structure whose mechanical and physiological responses to movement can be evaluated and influenced through targeted techniques [5, 6]. Biomechanical studies distinguish between two primary types of neurodynamic techniques: sliders and tensioners. Sliders enhance neural excursion with relatively low strain, whereas tensioners induce higher strain and require precise dosing in cases of heightened mechanosensitivity [7]. Clinical trials and systematic reviews support the efficacy of neural mobilization for “nerve-related” neck-shoulder and cervicobrachial symptoms, although population and protocol heterogeneity may influence the robustness of these conclusions [8–11]. The validity and reliability of clinical neurodynamic tests, such as the Upper Limb Neural Tests (ULNT), are essential for identifying nerve-related presentations and monitoring changes in mechanosensitivity [12, 13].

Against this background, a direct comparison between standard kinesitherapy (SK) and a neurodynamic (ND) program in office workers holds significant practical relevance for the prevention of chronic vertebrogenic musculoskeletal disorders.

Aim

To conduct a comparative analysis of the effectiveness of standard kinesitherapy (SK) versus a neurodynamic program (ND) in the prevention of chronic vertebrogenic musculoskeletal disorders among office employees.

Materials and methods

Study population and randomization

The present study was conducted with a cohort of 60 office employees, all of whom met the inclusion criteria of engaging in sedentary occupational tasks for a minimum of 6 hours per day. Following the initial screening, participants were allocated into two parallel intervention groups using a 1:1 randomization ratio: the Standard Kinesitherapy (SK) group (n = 30) and the Neurodynamic (ND) group (n = 30). To ensure the safety and homogeneity of the sample, the selection process strictly adhered to specific criteria designed to exclude individuals presenting with clinical “red flags”, severe radiculopathy, or established neurological deficits.

Assessment protocol and clinical metrics

Evaluations were systematically performed at two distinct intervals: at baseline (T0) and upon completion of the 12-week intervention period (T1). A multi-dimensional assessment approach was utilized, incorporating several validated clinical instruments. Pain intensity was quantified using the Numeric Pain Rating Scale (NPRS), while functional limitations related to cervical spine health were measured via the Neck Disability Index (NDI). To determine the clinical relevance of the observed changes, the Minimal Clinically Important Change (MCIC) was established using an anchor-based methodology. Additionally, participants provided self-reported data regarding their weekly frequency of pain (days per week). Neural mechanosensitivity was objectively assessed through the Upper Limb Neural Test (ULNT), where the range of motion (ROM) deficit at the point of symptom provocation was measured goniometrically – a method characterized by high validity and reliability in existing neurodynamic literature.

Therapeutic interventions

Both cohorts received a foundational ergonomic counseling session, which aimed to optimize the occupational environment. This instruction covered the strategic positioning of the monitor and peripheral devices, the utilization of forearm and lumbar supports, and the integration of scheduled micro-breaks to enhance postural variability.

The Standard Kinesitherapy (SK) protocol was structured as a hybrid model, consisting of two supervised sessions per week (45–60 minutes each) and a supplementary home-based program (15–20 minutes, 3–4 times weekly). The therapeutic focus of the SK group centered on enhancing postural control, achieving stable cervico-scapular and lumbopelvic alignment, and increasing overall flexibility and movement variability to counteract the physiological strain of prolonged static loading.

The SK intervention followed a systematic progression focusing on postural restoration and muscular balance (Table 1).

Table 1. Standard Kinesitherapy (SK) Protocol: 12-Week Phased Model

Phase	Primary Focus	Core Exercises	Volume and Progression
Weeks 1–4	Activation and Control	Craniocervical flexion; „chin tucks"; scapular retraction/depression; thoracic extension; "dead bug"; "glute bridge"; pectoralis minor/levator scapulae stretching.	2–3 sets × 8–12 reps; low resistance; emphasis on movement quality and technique.
Weeks 5–8	Strength and Endurance	Resistance bands: rowing, external rotation, "Y/T/W" patterns, "face pull"; modified side plank; hip-hinge; thoracic mobility drills.	3 sets × 10–15 reps; progressive increase in resistance and time under tension.
Weeks 9–12	Integration and Prevention	Combined movements (e.g., row + chin tuck); lunge with thoracic rotation; loaded carries; breath-movement coordination; individualized focus on dominant segments.	3–4 sets; RPE 5–6/10; development of a long-term maintenance plan.

The ND program was structured around the theoretical framework of neural tissue management [5–7]. The intervention consisted of daily home-based sessions (10–15 min, 4–6 days/week) integrated with two supervised weekly sessions. The progression logic followed a biomechanical sequence from sliders to tensioners [7].

The selection of the "primary" and "secondary" neural targets was algorithmically determined based on the participant's symptomatic profile and the most provocative Upper Limb Neural Test (ULNT) [12, 13] (Table 2).

Flare-up Protocol: In the event of a pain increase ≥ 2 points on the NPRS persisting for > 24 hours, participants were instructed to regress to Level A (sliders) for 7 days, reducing amplitude by 30–50% and frequency to every other day.

Statistical analysis. The primary intergroup effect at the 12-week follow-up (T1) was evaluated using Analysis of Covariance (ANCOVA), with T1 scores as the dependent variable, group allocation as the fixed factor, and baseline (T0) scores as the covariate (T1 ~ Group + T0). Effect sizes were quantified using partial eta squared (ηp^2) to determine the magnitude of the clinical impact.

Results

Baseline characteristics. At baseline (T0), no statistically significant differences were observed between the two groups regarding age, sex distribution, body mass index (BMI), occupational sitting time, or the segmental distribution of musculoskeletal complaints, confirming that the groups were well-matched for comparison (Table 3).

Table 2. Neurodynamic (ND) Program: Menu-Based Approach

Level	Condition	Exercise Templates	Dosage and Progression Criteria
Level A: Sliders	Weeks 1–4 or high mechanosensitivity	Median/ulnar/radial sliders (primary + secondary) with contralateral neck movement for neural unloading.	2 sets × 10–15 reps; discomfort ≤3/10; no symptom exacerbation > 24 h
Level B: Mixed	Stable symptoms for ≥7 days	Primary nerve tensioner combined with a secondary nerve slider (cool-down).	Tensioner: 2×6–10; Slider: 1×10–12; frequency 4–5 days/week.
Level C: Tensioners	Weeks 9–12 or maintenance phase	Dual tensioners (primary + secondary); optional slider if required.	2 sets × 6–10 reps; 3–5 days/week; rigorous monitoring of neural response.

Table 3. Baseline Participant Characteristics (T0)

Parameter	SK Group (n=30)	ND Group (n=30)
Age (years), mean ± SD	37.5 ± 6.1	36.9 ± 5.8
Sex (Female), n (%)	18 (60%)	17 (57%)
BMI (kg/m²), mean ± SD	24.7 ± 3.1	24.3 ± 3.0
Occupational Sitting (hours/day), mean ± SD	7.6 ± 0.9	7.5 ± 1.0
Primary Complaint Segment (Cervical/Thoracic/Lumbar), n	14/6/10	15/5/10

Primary outcomes (T0 to T1). Both intervention groups demonstrated longitudinal improvements across all clinical metrics. However, the Neurodynamic (ND) group exhibited a more pronounced reduction in pain intensity (NPRS), functional disability (NDI), and frequency of symptomatic days, alongside a greater decrease in ULNT ROM deficit compared to the Standard Kinesitherapy (SK) group (Table 4).

ULNT ROM is expressed as a deficit in degrees; higher values indicate poorer tolerance or more restricted range of motion during provocation.

Inter-group analysis (ANCOVA). The Analysis of Covariance (ANCOVA), adjusting for baseline values, revealed statistically significant inter-group differences at T1 in favor of the ND group across all primary outcomes. The most substantial effect sizes were observed for functional disability (NDI) and the frequency of pain days (Table 5).

Discussion

The results of this randomized controlled trial demonstrate that both Standard Kinesitherapy (SK) and the Neurodynamic (ND) program lead to significant clinical improvements over a 12-week period. these findings are consistent with existing evidence supporting the efficacy of structured exercise interventions for office-based populations [2–4]. However, the ND group achieved statistically superior outcomes compared to the SK group across all assessed metrics: pain intensity, functional disability, frequency of pain days, and neuromechanical tolerance (ULNT deficit). Notably, the strongest effect sizes were observed for the Neck Disability Index (NDI) and the number of symptomatic days ($\eta p^2 \approx 0.24–0.26$).

Table 4. Comparison of Pre- and Post-Intervention Outcomes (mean ± SD)

Outcome	Group	T0 (Baseline)	T1 (12 Weeks)	Δ (T1–T0)
NPRS (0–10)	SK	4.70 ± 1.57	3.10 ± 1.28	−1.60
	ND	4.46 ± 1.30	2.37 ± 1.27	−2.09
NDI (0–50)	SK	19.88 ± 6.19	17.17 ± 5.63	−2.72
	ND	19.77 ± 6.48	12.43 ± 5.12	−7.34
Pain Days (0–7)	SK	3.69 ± 1.95	2.69 ± 1.49	−1.00
	ND	3.63 ± 1.93	1.40 ± 1.77	−2.23
ULNT ROM Deficit (°)*	SK	17.77 ± 4.63	12.36 ± 4.93	−5.41
	ND	18.15 ± 6.30	8.95 ± 5.80	−9.19

Table 5. Inter-group Treatment Effects (ANCOVA: T1 ~ Group + T0)

Outcome	Adjusted Difference (SK–ND) at T1	95% CI	p-value	ηp2
NPRS	0.58	0.10 до 1.06	0.019	0.092
NDI	4.68	2.45 до 6.91	< 0.001	0.236
Pain Days	1.25	0.69 до 1.82	< 0.001	0.258
ULNT Deficit	3.63°	1.39 до 5.86	0.002	0.156

The more pronounced reduction in ULNT ROM deficit within the ND group is clinically significant. Upper Limb Neural Tests are established tools for identifying neural components in neck-shoulder and cervicobrachial presentations and for monitoring longitudinal changes in mechanosensitivity [12, 13]. The underlying mechanism of the ND intervention aligns with current concepts of mechanical and physiological "management" of neural tissue [5, 6]. Furthermore, the phased sliders – tensioners approach is supported by biomechanical data suggesting that sliders enhance neural excursion at lower strain-making them ideal for high mechanosensitivity – whereas tensioners induce higher strain and require precise, progressive dosing [7].

Current systematic reviews on nerve-related cervical disorders and cervicobrachial pain report favorable effects of neural mobilization but highlight variability in evidence quality and the necessity of precise subgroup definition [9–11]. This underscores the importance of the menu-based algorithm used in this study, which allows for individualization based on the participant's symptomatic profile and their specific ULNT reactivity. Conversely, some randomized trials have suggested that adding specific neural mobilization to other manual techniques may not always yield additional benefits, reinforcing the premise that population selection and dosage are decisive factors [8]. In an occupational context, the combination of ergonomic modifications and structured exercise remains fundamental [2–4], and neurodynamics may represent a rational adjunct for individuals with marked neuromechanical sensitivity.

A primary limitation of the current design is the absence of long-term follow-up at 3 and 6 months (T2/T3). Such data would allow for an assessment of the sustainability of the therapeutic effect in a sedentary population, where regression is frequently observed in the absence of a maintenance program [4].

Conclusion

Both standard kinesitherapy and the neurodynamic program are effective interventions for the prevention and reduction of symptoms in office workers with vertebrogenic musculoskeletal complaints over a 12-week period. However, the neurodynamic intervention demonstrated statistically significant superior improvements compared to standard kinesitherapy regarding pain intensity (NPRS), functional disability (NDI), frequency of symptomatic days, and neuromechanical tolerance (ULNT deficit).

These data support the implementation of an individualized, menu-based neurodynamic protocol (sliders – tensioners) as a valuable complementary strategy to standard postural stabilization and ergonomic prophylaxis for office-based populations.

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The HL7 standard and its application in Bulgarian healthcare information systems

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Standardization in healthcare ensures high-quality care, efficiency, and data security. It provides unified formats and protocols for exchanging medical data between different hospital systems and institutions. The HL7 standard is one of the most widely used international standards for exchanging, integrating, and managing health information. HL7 was specifically created to enable multiple healthcare systems to communicate in a uniform and interoperable manner, regardless of their technical or organizational characteristics, by defining both semantic and structural requirements for data exchange. In Bulgaria, HL7 is utilized for accessing electronic health records, issuing and validating electronic prescriptions, creating pharmaceutical records, automatically transmitting laboratory and imaging results to a hospital information system, and integrating various modules within a single medical institution. HL7 forms the foundation of the European Health Area, which aims to facilitate the cross-border exchange of medical expertise, ensuring the highest level of healthcare, and generally enhancing the qualifications of medical personnel.
Keywords: *HL7 standard, electronic health records, healthcare information systems, hospital information system, medical institution*

Introduction

Contemporary healthcare depends on the timely exchange of reliable clinical and administrative information. In practice, however, data are still produced and stored in heterogeneous systems: hospital information systems, laboratory platforms, radiology systems, pharmacy modules, registries, and national electronic health services. When these systems use different structures and terminologies, the same clinical fact may be interpreted differently or transferred manually, which increases the risk of errors and delays.

The Health Level Seven (HL7) family of standards was developed to address this problem by defining a common approach to exchanging health information at the application level of the Open Systems Interconnection model [1]. Internationally, HL7 is used for laboratory results, admission-discharge-transfer messages, electronic health records, electronic prescriptions, clinical documents, and integration between institutional and national platforms [2, 3].

In Bulgaria, the need for interoperability is especially visible because new central services coexist with local systems developed at different times and by different vendors. The National Health Information System, electronic prescriptions, electronic referrals, and access to patient records create a basis for digital healthcare, but they do not automatically solve the problem of semantic and technical compatibility. For this reason, HL7, and particularly Fast Healthcare Interoperability Resources (FHIR), should be considered both a technical and strategic priority.

Aim

The aim of this review is to present the nature, evolution, and practical application of the HL7 standard, with emphasis on its relevance to Bulgarian healthcare information systems, the main barriers to implementation, and the expected benefits for clinical, administrative, and telemedicine processes.

Methods

A narrative literature review was performed. The analysis included official HL7 materials, specifications and descriptions of HL7 version 2.x, HL7 version 3, Clinical Document Architecture, and HL7 FHIR, as well as peer-reviewed publications on interoperability, electronic health records, clinical document exchange, and digital health infrastructure [1–10]. Publicly available information related to digital health policy, the European Health Data Space, and the Bulgarian healthcare environment was also reviewed [11–13].

The collected information was synthesized thematically in several areas: definition and evolution of HL7; major versions and related standards; areas of application; current and potential use in Bulgaria; barriers to implementation; expected benefits; and future perspectives in the context of European digital health policy.

Results

Definition and significance of HL7. HL7 is a set of international standards used for the exchange, integration, sharing, and retrieval of electronic health information. Its practical value is that it provides a common language between systems that would otherwise require individual, vendor-specific interfaces. In a hospital environment, this means that the laboratory, pharmacy, radiology, administrative, and hospital information systems can exchange structured data instead of relying on repeated manual entry or informal file transfers.

Evolution of the standard. HL7 version 2.x remains the most widely used implementation in many healthcare institutions. It is based on text messages divided into segments and fields, which makes it flexible and relatively easy to adapt to local workflows [3]. This flexibility explains its long-term use, especially for laboratory results, admission and discharge events, billing, pharmacy orders, and internal hospital integrations. At the same time, local adaptation may lead to institution-specific variations and can complicate national interoperability.

HL7 version 3 introduced a more formal information model and stronger semantic control, but its complexity limited its routine adoption [4]. Nevertheless, the Clinical Document Architecture became an important result of this approach and remains relevant for structured clinical documents [5]. HL7 FHIR represents the most modern direction in the development of the standard. It uses resources such as Patient, Observation, MedicationRequest, Encounter, and DiagnosticReport, and is compatible with contemporary web technologies, including representational state transfer interfaces, JavaScript Object Notation, and Extensible Markup Language [2, 6]. This makes FHIR more suitable for mobile applications, telemedicine, patient portals, and decision-support tools [7–9].

Areas of application. HL7 is used in clinical practice for patient demographic data, diagnoses, observations, treatment plans, medication orders, discharge summaries, and clinical documents. In laboratory medicine, it enables automatic transmission of results from laboratory systems to hospital or outpatient systems. In pharmacy workflows, it supports medication requests, electronic prescriptions, dispensing data, and pharmaceutical records. In diagnostic imaging, HL7 is commonly used together with Digital Imaging and Communications in Medicine, where imaging objects are managed through DICOM and the surrounding clinical communication is supported through HL7 [14].

Application in Bulgarian healthcare information systems. In Bulgaria, electronic healthcare has developed significantly in

recent years, but the environment remains partly fragmented. The National Health Information System provides a central infrastructure for electronic health records, electronic prescriptions, electronic referrals, and other digital services. At the same time, hospitals, laboratories, pharmacies, and outpatient practices often continue to use systems with different architectures, internal data models, and levels of readiness for standard-based integration.

In this context, HL7 can support the automatic transfer of laboratory results to hospital information systems and electronic patient records, improve the connection between prescribing and dispensing systems, assist integration between hospital modules, and provide a more stable basis for communication between local providers and national electronic health services. The Bulgarian case shows that the presence of a national system alone is not sufficient. Interoperability also requires implementation guides, technical profiles, consistent terminology, testing procedures, certification mechanisms, and trained specialists.

Barriers to implementation. The main barrier is the lack of a unified implementation approach across institutions. Each hospital, laboratory, pharmacy, or software vendor may interpret integration requirements differently. A second barrier is the limited number of specialists who understand both the technical structure of HL7 and the clinical-administrative logic of the Bulgarian healthcare system. Financing is also significant, because standard-based interoperability is often perceived as an additional cost, although its benefits are realized later through fewer errors, reduced duplication, faster workflows, and better data availability. Cultural and organizational resistance should also be considered, since new digital processes may be seen as administrative burden if they are not designed around real clinical workflows.

Benefits of HL7 implementation. The first direct benefit is improved communication between healthcare institutions. When data from general practitioners, laboratories, hospitals, pharmacies, and national systems can be exchanged in a structured way, clinicians receive a more complete view of the patient. Another benefit is the reduction of errors related to manual transcription of laboratory results, medication data, demographic information, and diagnostic reports. HL7 FHIR also supports telemedicine and remote monitoring by enabling exchange of observations from devices, mobile platforms, and patient portals. Finally, structured health data can improve hospital management, regional planning, research, public health surveillance, and secondary use of data, provided that the information is collected consistently and with appropriate semantic standards.

Discussion

The implementation of HL7 in Bulgaria should be interpreted as a gradual process rather than as a single technical upgrade. The country already has important digital health components, but full interoperability requires alignment between local systems, national platforms, legal requirements, and European data exchange initiatives. The main challenge is not whether HL7 can technically be used, but whether it can be implemented in a coordinated and sustainable way.

A practical strategy should combine several layers. At the technical level, HL7 FHIR should be prioritized for new services because it is compatible with modern software development and patient-oriented applications. At the same time, HL7 version 2.x should continue to be supported where

legacy systems are already operational, especially in laboratories and hospitals. This mixed approach is more realistic than expecting an immediate replacement of existing infrastructure.

At the semantic level, HL7 should not be applied in isolation. True interoperability requires integration with terminologies and standards such as Systematized Nomenclature of Medicine Clinical Terms, Logical Observation Identifiers Names and Codes, International Classification of Diseases, and DICOM [14, 15]. Without shared terminologies, systems may exchange messages successfully but still interpret clinical meaning differently.

At the organizational level, Bulgaria needs national implementation guides defining how HL7 should be used for specific scenarios: laboratory results, electronic prescriptions, electronic referrals, discharge summaries, imaging reports, and access to electronic health records. Training is also essential. Medical informatics should be strengthened in the education of healthcare professionals, software developers, and health managers, because interoperability projects require cooperation between clinical and technical specialists.

The European Health Data Space adds an additional reason to accelerate standardization. Cross-border exchange of health data and secondary use of data for research and public health will require structured, secure, and semantically consistent information [11]. If Bulgaria develops national HL7 profiles early, it will be better prepared to participate in European digital health services and to support innovation in telemedicine, artificial intelligence, and personalized medicine.

Conclusion

The HL7 standard is one of the key instruments for achieving interoperability in contemporary healthcare information systems. Its evolution from message-based HL7 version 2.x, through the more formal version 3 and Clinical Document Architecture, to resource-based HL7 FHIR reflects the growing need for flexible, secure, and semantically meaningful exchange of health information.

For Bulgaria, the implementation of HL7 is both a technical necessity and a strategic opportunity. The development of national electronic health services, electronic prescriptions, electronic records, and digital communication between institutions creates a foundation for broader standardization. However, this process requires coordinated governance, national implementation guides, investment in infrastructure, and systematic training of specialists.

The consistent adoption of HL7, especially HL7 FHIR for new digital health services, can improve patient safety, reduce administrative duplication, support telemedicine, and create better conditions for research, management, and participation in the European Health Data Space. The standard should therefore be regarded as a core element of healthcare modernization in Bulgaria.

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Investigation of the relationship between the Tiffeneau index and body mass index among volunteers

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Introduction: The establishment of changes in respiratory function and normal body weight is of significant medical importance for the prevention and treatment of socially significant diseases. An increasing number of people without diagnosed respiratory pathology exhibit obstructive ventilatory disorders attributable to generalized obesity. Common complications of obesity include respiratory and cardiovascular diseases. **Purpose:** The present study examines the relationship between two parameters – the Tiffeneau index (FEV_1/FVC ratio) and BMI (Body Mass Index). **Materials and methods:** The study was conducted in two stages among nine volunteers of both sexes aged 17–19 years. It begins with the calculation of the indices using spirometry and anthropometric measurements. During the second stage, participants are subjected to physical exertion, during which their average heart rate is recorded using specialized measuring device. **Results:** In three participants, anamnestic data reveal respiratory complaints, a reduced Tiffeneau index, and evidence of mild airway obstruction, while four participants demonstrate increased BMI values. In one volunteer, a proportional relationship between the two parameters is observed. Higher heart rate values are reported both in individuals with increased BMI and in those with reduced Tiffeneau index values. **Discussion and conclusion:** The results indicate that, in order to confirm or reject the primary hypothesis, a number of studies among individuals with obesity under specific conditions and physical load is required.
Keywords: *Tiffeneau index, BMI, respiratory function, obesity*

Introduction

Modern society is characterized by an increasing prevalence of lifestyle-related diseases, among which obesity and chronic respiratory disorders occupy a prominent place. The identification of alterations in respiratory function and deviations in body composition is of substantial importance for the early prevention, diagnosis, and treatment of a wide range of socially significant diseases. In recent years, there has been a growing tendency toward the detection of functional respiratory impairments in young individuals without diagnosed pulmonary pathology, particularly in association with elevated body mass index (BMI). The accumulation of adipose tissue, especially in the thoracic and abdominal regions, restricts diaphragmatic mobility, reduces pulmonary capacity, and increases airway resistance, potentially contributing to the development of obstructive respiratory dysfunction.

One of the principal indicators used in the assessment of pulmonary function is the Tiffeneau index (FEV_1/FVC), defined as the ratio between the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FVC). This parameter possesses considerable diagnostic value in identifying impairments in airway patency and is widely utilized in clinical practice for the early detection of obstructive respiratory diseases. In parallel, body mass index (BMI) represents a readily applicable anthropometric indicator employed for the evaluation of nutritional status and the risk of metabolic and cardiovascular complications.

Current scientific evidence suggests that obesity exerts a detrimental effect on respiratory mechanics, gas exchange, and the adaptive capacity of the organism during physical exertion. Individuals with elevated BMI frequently demonstrate reduced spirometric parameters, increased heart rate, and diminished physical endurance. Nevertheless, the relationship between the Tiffeneau

index and BMI in young individuals without diagnosed chronic diseases remains insufficiently investigated.

Particular interest is associated with the assessment of these parameters under conditions of physical load, as exercise reveals the actual adaptive and functional capacity of the organism. Monitoring heart rate during exertion provides additional information regarding physiological stress and the efficiency of cardiorespiratory adaptation. This enables a more precise evaluation of the risk of latent functional impairments that may remain undetected under resting conditions.

The present study aims to investigate the relationship between the Tiffeneau index and body mass index in young healthy volunteers, as well as to evaluate the influence of these parameters on the physiological response of the organism during physical exertion.

Purpose

The aim of the present study is to investigate the relationship between the Tiffeneau index (FEV_1/FVC) and body mass index (BMI) in young volunteers, as well as the influence of these parameters on the physiological adaptation of the organism during physical exertion.

Materials and methods

The study was conducted in two consecutive stages involving nine volunteers of both sexes, aged between 17 and 19 years. All participants were previously informed about the aims and methodology of the study and participated voluntarily.

During the first stage, a comprehensive anthropometric and functional assessment of the participants was performed. Height, body weight, and chest circumference were measured, after which body mass index (BMI) was calculated using the standard formula. The obtained values were interpreted according to the World Health Organization criteria for the assessment of nutritional status.

In parallel with the anthropometric measurements, spirometric testing was performed to evaluate respiratory function. Standard spirometry was used to determine forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Based on these parameters, the Tiffeneau index (FEV_1/FVC) was calculated. The Tiffeneau index was used as the primary criterion for assessing airway patency and the presence of potential obstructive respiratory disorders.

During the first stage, anamnestic data were also collected regarding previous respiratory diseases, infections, bronchitis, allergic manifestations, and smoking habits. These data were taken into consideration during the interpretation of the functional results.

The second stage of the study involved the performance of physical exercise, during which the physiological response of the organism was monitored. Participants were assessed using specialized Garmin Vivo monitoring devices, which enabled the recording of average heart rate (HRavg), maximal heart rate (HRmax), and heart rate variability parameters. These indicators were used to evaluate the degree of cardiorespiratory adaptation during physical exertion.

A comparative analytical approach was applied for the processing and analysis of the obtained results, enabling the evaluation of the relationships between BMI values, the Tiffeneau index, and cardiovascular parameters during physical exertion.

Results

The analysis of the anthropometric data demonstrated that four of the participants presented with elevated body mass index (BMI) values, ranging from overweight to

severe obesity. The highest BMI value was recorded in participant No. 3 – 43.5 kg/m², corresponding to severe obesity. The remaining participants were within normal or borderline BMI ranges.

Spirometric assessment revealed normal pulmonary function parameters in six of the volunteers. In three participants – № 5, № 6, and № 7 – reduced Tiffeneau index values were registered, specifically 73.5%, 74.7%, and 70.9%, respectively, corresponding to a mild degree of obstructive airway impairment. In these participants, anamnestic data indicated the presence of respiratory complaints and previous respiratory conditions, including respiratory hyperreactivity, acute rhinitis, and a history of bronchitis.

The obtained results indicate that respiratory dysfunction was not observed in all participants with elevated BMI. For example, participant No. 3 demonstrated a markedly increased BMI but no evidence of obstructive changes in spirometric parameters. Conversely, participant No. 7 exhibited mild obstructive impairment at a BMI of 26.0 kg/m², suggesting that respiratory dysfunction may also be influenced by factors other than body composition alone. Of particular interest is the observation that one volunteer demonstrated a relatively proportional relationship between the Tiffeneau index and BMI, whereas no clear correlation was identified among the remaining participants. These findings suggest the presence of a complex interaction between body composition, pulmonary function, and the adaptive mechanisms of the organism.

Participants with normal spirometric findings and favorable heart rate variability parameters demonstrated a more efficient physiological adaptation to physical exertion, further confirming the importance of optimal respiratory function for maintaining adequate physical performance capacity.

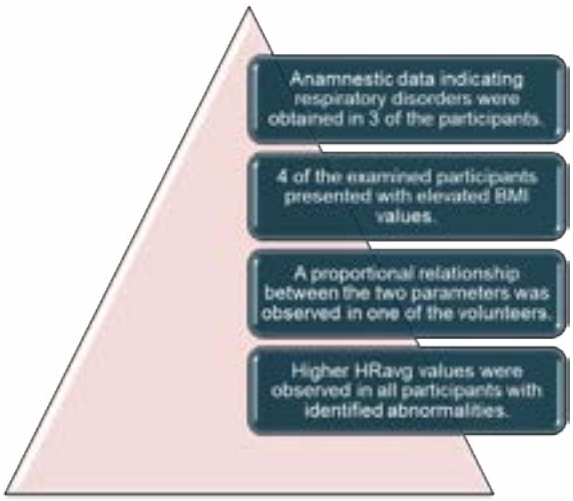

Figure 1. Summary findings and conclusions

Discussion

The obtained results confirm the importance of functional assessment of the respiratory system in young individuals, including those without diagnosed chronic diseases. Despite the limited number of participants, the study demonstrated the presence of respiratory abnormalities in some of the volunteers that could not have been identified solely through subjective evaluation of physical condition.

Reduced Tiffeneau index values observed in three of the participants indicate the presence of mild airway obstruction. In two cases, the probable cause was previous acute inflammatory respiratory processes, whereas in the third participant the findings were associated with respiratory hyperreactivity. These results emphasize the necessity for regular monitoring of pulmonary function in young individuals, particularly in the presence of anamnestic data indicating previous respiratory disorders.

The study did not establish a definitive linear relationship between body mass index and the Tiffeneau index. Although some participants with elevated BMI demonstrated functional respiratory abnormalities, others showed no impairment in spirometric parameters. This suggests that BMI alone is not

a sufficient predictor of respiratory dysfunction. Multiple additional factors are likely to contribute, including physical activity level, muscle mass, previous infections, smoking habits, and individual adaptive mechanisms.

Particular significance is associated with the findings related to heart rate during physical exertion. Participants with reduced Tiffeneau index values demonstrated a tendency toward higher average heart rate (HRavg) values and lower heart rate variability (HRV). These findings suggest the presence of increased physiological stress and reduced efficiency of autonomic regulation. Reduced HRV is widely regarded in the scientific literature as an indicator of limited recovery capacity and increased sympathetic nervous system activity.

The results further demonstrate that an apparently good physical condition and normal body composition do not necessarily guarantee optimal internal functional capacity. Some participants with normal BMI exhibited respiratory abnormalities, whereas others with elevated BMI showed no significant functional impairment. These findings highlight the need for a comprehensive approach to the assessment of physical condition, incorporating both anthropometric and functional investigations.

Conclusion

The study confirms the necessity of implementing spirometric and cardiophysiological assessments as part of early prevention strategies and the evaluation of physical fitness in young individuals. The combined assessment of respiratory function, body composition, and cardiac adaptation provides a more comprehensive understanding of the organism's functional capacity and enables the timely identification of latent physiological abnormalities. Given the limited number of participants, additional studies involving larger sample sizes are required in order to confirm or refute the main hypothesis. Future investigations should include individuals with varying degrees of obesity and different levels of physical activity. Conducting further studies under standardized conditions and at different levels of physical exertion would allow a more precise evaluation of the relationship between BMI, the Tiffeneau index, and the adaptive capacity of the organism.

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Survey of attitudes among medical students regarding vaccination against COVID-19

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Vaccination against COVID-19 has become established as a key measure for the control and prevention of the spread of the disease worldwide. Medical professionals have a key role both in the implementation of vaccines and in the formation of public attitudes towards immunization. **Objective:** to study the attitudes, awareness and attitude of medical students towards vaccination against COVID-19, identifying the factors influencing their choice and trust in vaccines. Materials and methods: an online questionnaire was developed and reached the respondents through student online groups. 189 students from the first to the sixth year of English-language medical education at MU-Plovdiv were included. The study period was October–December 2025. **Results:** men were 29.1% (n = 55) and women were 70.9% (n = 134). 9.0% (n = 17) reported suffering from chronic diseases, and 48.1% (n = 91) reported that their relatives had chronic diseases. Of these, 68.8% (n = 134) reported having recovered from Covid-19. 83.6% (n = 158) had followed all recommendations to prevent its spread, but 15.3% (n = 29) had only partially followed them. 89.9% (n = 170) reported being vaccinated, and 10.1% (n = 19) had not been vaccinated. **Conclusion:** The results of the study show a generally positive attitude among medical students towards vaccination against COVID-19. The majority of participants demonstrated a good level of awareness, confidence in the effectiveness and safety of vaccines, as well as an awareness of the role of immunizations in protecting public health. Medical students are future health professionals who are key to building trust in vaccination prevention among the population. Ongoing training and discussions on the topic are needed.

Keywords: *vaccination, Covid-19, prevention*

1. Introduction

COVID-19, caused by the SARS-CoV-2 virus, emerged in Wuhan, China, in December 2019 and rapidly spread worldwide, with the WHO declaring it a global pandemic [1]. The virus primarily attacks the respiratory system, making vaccination an essential measure for controlling transmission and reducing disease severity [2].

Although COVID-19 vaccination has been widely implemented and has played a crucial role in controlling the pandemic globally, vaccine hesitancy remains prevalent. In 2019, the World Health Organisation identified vaccine hesitancy as one of the ten major threats to global health [3]. For this reason, our research aimed to examine the attitudes of medical students toward vaccines, as they represent future healthcare professionals who play a key role in promoting public health, influencing patient decisions, and supporting vaccination programs [4]. Their knowledge, perceptions, and acceptance of vaccines can directly affect vaccine confidence and healthcare practices. Therefore, assessing their attitudes can help identify gaps in knowledge and guide effective strategies to better educate students so they can accurately inform future patients and reduce vaccine hesitancy.

The study aims to assess attitudes towards the COVID-19 vaccine and the willingness to receive vaccination among medical students at the Medical University of Plovdiv. It expands on existing research by evaluating the impact of knowledge and awareness of students and their attitudes toward COVID-19 vaccines [5, 6]. Although previous studies suggest that attitudes toward COVID-19 vaccination may improve over time as vaccine uptake increases and its benefits become more apparent, hesitancy and refusal

continue to be reported in certain groups, including students [7]. Among medical students, these attitudes may be influenced by sociocultural and socioeconomic factors, which can shape perceptions of vaccine safety, effectiveness, and acceptance.

2. Objective

Our objective is to assess medical students' awareness, attitudes, and perceptions regarding COVID-19 vaccination, and to identify the factors influencing their decision-making and level of trust in vaccines

3. Materials and methods

3.1. Study design

A cross-sectional survey study was conducted using an online questionnaire. The survey was distributed through messaging platforms and via QR code access, completed by students from the first to the sixth year of English-language medical education at MU-Plovdiv. The student respondents included Faculty of medicine and Faculty of dental medicine. The study period was carried out between October – December 2025

3.2. Questionnaire

Our questionnaire consisted of 30 questions divided into four sections. The first section collected personal information. This information was used to categorise participants and identify possible variables, such as age, gender, or speciality, that may have influenced the responses given in the study. The second section focused on attitudes toward COVID-19 and aimed to understand participants' experiences with the disease and their perceptions of COVID-19 in the post-pandemic period. Questions included whether they had previously contracted COVID-19, how many times they had been infected, and the risk assessment. The third section explored vaccine history and attitudes. Participants were asked whether they had received the COVID-19 vaccine, whether they experienced any side effects, and whether they had received booster doses. Open-ended questions were also included to allow participants to explain their reasons for not receiving the vaccine and/or booster doses. The final section examined how participants' attitudes toward COVID-19 vaccination might influence their future professional practice. Questions included whether they would recommend the vaccine to future patients and whether they would be willing to receive a newly developed vaccine in the future.

4. Results

4.1. Sample description

By the end of the study, a total of 189 responses were recorded. The distribution of participants from the English language medical education at MU-Plovdiv is illustrated in Figure 1. Gender distributions of our survey were unbalanced, with the majority of the participants being women 70.9% (n = 134). According to distributions based on faculty, 87.8% (n = 166) were medical students and 11.2 % (n = 21) being dental medical students. The average age of students is 22.33 ± 2.559

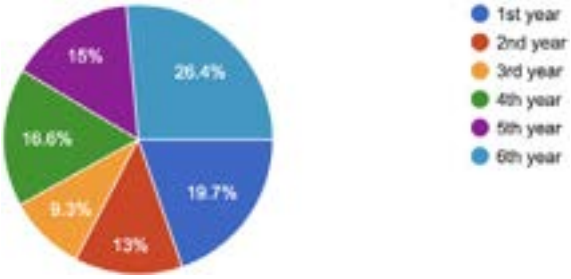

Figure 1. Distribution of participants according to their course of study

4.2 Attitudes towards vaccine

Overall, the students demonstrated a generally positive attitude toward the COVID-19 vaccine, with 89.2% (n = 170) reporting

they had received the initial vaccination. However, this proportion decreased notably when booster uptake was assessed, with only 67.9% (n = 128) confirming they had received a booster dose.

4.3. COVID-19 knowledge and future vaccinations

Participants self-evaluated their knowledge of COVID-19, with only 6.1% (n = 12) reporting low confidence. When asked whether they would recommend the COVID-19 vaccine to their future patients, 62.8% (n = 119) of participants stated that they would confidently recommend it. In response to a separate question, participants were asked how likely they would recommend an updated or new variant of the COVID-19 vaccine. Overall, 40.8% (n = 77) reported being very likely to receive the vaccine, while 18.9% (n = 36) stated they were very unlikely to do so, and 40.3% (n = 76) were unsure.

4.4. Vaccine safety

Participants were asked to rate the safety of vaccines in general as well as the safety of the COVID-19 vaccine on a scale from 1 to 5. 45.4% (n = 86) rated vaccines in general as 5 (very safe), and 15.8% (n = 30) assigned a score of 3 or lower. In comparison, when participants rated COVID-19 vaccine, only 26.6% (n = 50) rated 5 and a substantial proportion 41.9% (n = 79) assigned a score of 3 or lower. The main reasons reported were concerns that the vaccine was not adequately studied, exposure to conspiracy-related information, and the lack of trust in governmental institutions. Despite the reported concerns regarding the COVID-19 vaccine, 62.8% (n = 119) of participants stated that they would recommend the vaccine to their patients.

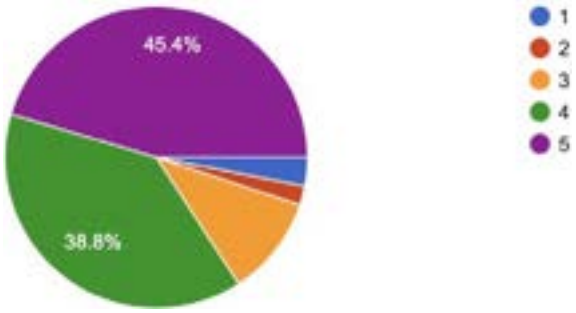

Figure 3. Distribution of participants according to how safe they feel vaccines in general are with 1 being the lowest and 5 the highest

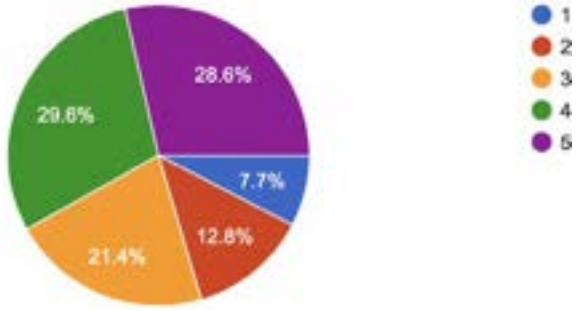

Figure 4. Distribution of participants according to how safe they feel the COVID-19 vaccine is with 1 being the lowest and 5 the highest

5. Discussion

The study aims to assess attitudes towards the COVID-19 vaccine among medical students at the Medical University of Plovdiv. Overall, students demonstrated a positive attitude, with 89.2% receiving the initial vaccine, and 69% received a booster dose. Moreover, 62.8% of students would confidently recommend the COVID-19 vaccination to their future patients. Additionally, the study identified several factors that altered attitudes, indicating that underlying perceptions and beliefs play a key role in shaping vaccine hesitancy among medical students.

When compared with the inter-university study conducted among medical students from Sofia, Varna, and Trakia Medical Universities in Bulgaria [5], the findings show broadly similar patterns of COVID-19 vaccine attitudes. Studies reveal that students have a positive attitude towards the Covid

vaccine, as well as similar reasons for hesitancy, particularly mistrust [5, 8, 9]. Evidence from their research [5] shows that vaccine acceptance is strongly shaped by trust in health authorities and reliance on credible scientific information, while misinformation and social media contribute to hesitancy. Students demonstrated higher vaccination uptake compared to the general population. However, the study did not fully investigate the difference in attitudes among medical students and overlooked the diverse and specific reasons for vaccine hesitancy among medical students [9]. Despite assumptions of more positive vaccination attitudes due to their background, medical students may still demonstrate hesitancy. Addressing these differences is essential to improve vaccine acceptance and reduce public mistrust, as disparities among medical colleagues could contribute to broader vaccine hesitancy. Understanding these factors is essential for developing effective strategies to improve vaccine acceptance, strengthen confidence in healthcare systems, and reduce public mistrust and vaccine hesitancy.

In our study, students demonstrated hesitancy towards receiving the vaccine. Although many students had received the initial vaccine, a common reason reported was that it was mandatory. When asked about their reasons for not receiving the booster ,which most responded was voluntary, many participants indicated that they believed the initial vaccination was sufficient. Others expressed concerns regarding the rapid development of the vaccines, as well as a perception that they were not at high enough risk to justify an additional dose. These responses suggest the presence of gaps in understanding of the role of the booster and also the transmissibility and virulence of COVID-19, particularly among vulnerable risk groups, if herd immunity is not achieved. Furthermore, when participants were asked to rate the COVID-19 vaccine on a scale from 1 to 5, a substantial proportion (41.9%) assigned a score of 3 or lower. The most commonly cited reasons included the belief that the vaccine had not been sufficiently studied, exposure to conspiracy-related information, and a general lack of trust in governmental institutions. When compared to the safety of vaccines in general, only 15.8% gave a score of 3 or lower, showing distrust, maybe limited to the COVID-19 vaccine alone and not to vaccines in general.

Similar to other cross-sectional survey studies, several limitations should be acknowledged.

The use of an online self-report questionnaire may have resulted in response bias as participants may provide socially acceptable answers. Moreover, the study was conducted at a single institution with a relatively limited sample size; the findings may not fully represent other medical student populations. The cross-sectional design only reflects participants' attitudes and opinions at a single point in time, meaning changes in perceptions over time and cause-and-effect relationships could not be evaluated. Furthermore, voluntary participation may have resulted in selection bias, as students with stronger opinions about COVID-19 vaccination may have been more likely to participate.

Conclusion

In conclusion, results from the study show a generally positive attitude among medical students towards the COVID-19 vaccine. The majority of participants demonstrate a good level of knowledge and awareness regarding vaccine effectiveness and safety. A strong understanding is essential as vaccinations play a crucial role in public health, as medical students are future

health professionals who are key to building trust in vaccination prevention among the population. Therefore to improve long-term vaccine confidence, regarding the COVID-19 vaccine and booster doses, targeted educational strategies should be strengthened to counteract the misinformation students displayed in the study towards the need for the vaccine. Clear, evidence-based information about vaccine safety, development, and long-term monitoring should be provided. It is also important to emphasize that although the vaccines were developed rapidly to deal with the pandemic, all essential safety and testing procedures were followed. Communication strategies should focus on transparency by honestly acknowledging uncertainties and directly addressing common myths and concerns, such as fears about safety or beliefs that vaccines were "not studied enough." Rather than using an overly authoritative approach, communication should encourage open dialogue and trust. In addition, trusted individuals such as healthcare professionals, university lecturers, and peer educators should be involved, as people are generally more likely to trust familiar and credible sources than institutions alone. Finally, misinformation on social media should be actively countered through accessible and shareable educational content, including infographics and short videos, while also teaching students how to critically evaluate the reliability of information sources.

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Effectiveness of Pilates-based programs in the rehabilitation of chronic low back pain

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Chronic low back pain (CLBP) is a significant medico-social problem associated with reduced functionality and quality of life. Conventional rehabilitation approaches often show limited long-term effectiveness, leading to increased interest in Pilates-based stabilization methods that emphasize torso activation, postural control, and neuromuscular coordination. **The aim** of the present study was to evaluate the effectiveness of a Pilates-based rehabilitation program on pain intensity, functional capacity, and postural control in patients with chronic nonspecific low back pain. **Material and methods:** The study included 20 patients with chronic nonspecific lumbar pain lasting more than 3 months in the period May–August 2025. Participants underwent a 16-week Pilates-based rehabilitation program, conducted 2–3 times a week under the supervision of a rehabilitation therapist. Assessment was performed using a visual analogue scale (VAS) and functional stability tests. Statistical analysis was performed using comparative methods with significance set at $p < 0.05$. **Discussion and results:** Significant reductions in pain intensity and functional impairment were observed after the intervention. The Pilates-based program demonstrated a positive effect on lumbar segment stabilization and motor control. **Conclusion:** Pilates-based rehabilitation programs are an effective, safe, and clinically applicable therapeutic approach for the management of chronic low back pain. Their integration into standard rehabilitation protocols may improve long-term outcomes, functional independence, and quality of life in patients with chronic nonspecific low back pain.

Keywords: *Pilates, rehabilitation, low back pain*

Introduction

Chronic low back pain (LBP) is among the most prevalent musculoskeletal disorders worldwide, significantly impacting quality of life and individual functional capacity [1]. Epidemiological data indicate [2] that the incidence of these complaints peaks in middle age, remaining a leading cause of disability and a serious socio-economic burden. According to the Years Lived with Disability (YLDs) report, low back pain ranks sixth among the ten leading causes of impaired quality of life and years lived with disability [3]. The WHO Global Burden of Disease (GBD) analysis reported that in 2021, the global prevalence of LBP increased from 7.3% to 12%. Significant geographical variations are observed, with the highest prevalence in the Russian Federation (56%) and the lowest in China (22%). In Bulgaria, 2021 data show a 25.73% prevalence of chronic non-specific LBP among the population over 60 years of age [4]. Back and lumbar pain are more common in women than men across all age groups, with frequency increasing after puberty [5].

Therapeutic Approaches Increasingly, physical exercises within the framework of WHO comprehensive rehabilitation programs are established as a leading therapeutic strategy for chronic lumbar pain [1, 4]. Non-specific lumbar pain is characterized by the absence of a clearly identifiable structural cause and is often associated with dysfunction of the spinal stabilizing musculature. Impaired control of the deep core muscles, including the deep abdominal muscles *(m. transversus abdominis* and *m. multifidus)*, leads to decreased stability of the lumbar segment and increased loading on the passive spinal structures.

There is significant interest in Pilates-based programs because they focus on activating and strengthening the so-called “muscular corset” – abdominal, back, and gluteal muscles – while improving posture and core stability. Research by Patti et al. (2024), Lim et al. (2024), and Yamato et al. (2023)

demonstrates that dysfunction of the deep stabilizing muscles plays a vital role in the development and persistence of chronic low back pain [6, 7, 8].

Pilates-based therapy. Conventional rehabilitation approaches often show limited long-term efficacy, stimulating interest in alternative therapeutic strategies. One modern method is Pilates-based therapy, which emphasizes core stabilization, postural control, and neuromuscular coordination improvement. Due to these characteristics, Pilates exercises are widely used in the rehabilitation of patients with chronic lumbar pain. The goals of these exercises are to improve joint range of motion, strengthen the musculature, increase functional ability, and enhance the quality of life. Current assessments suggest that Pilates-based programs improve general well-being, movement confidence, and the ability to perform daily activities, positioning them as an effective component of a multidisciplinary rehabilitation approach.

Aim

The aim of the present study is to evaluate the effectiveness of a Pilates-based rehabilitation program on pain intensity and functional capacity in patients with chronic non-specific low back pain.

Material and methods

The study included 20 patients with chronic non-specific lumbar pain lasting more than three months, examined between May and August 2025 during Pilates-based rehabilitation programs. Patient assessment was performed using:

- Visual Analog Scale (VAS) for pain intensity;
- Functional tests for core stability;
- Clinical assessment of functional capacity.

Statistical analysis was conducted through a comparative analysis of results before and after the intervention.

Inclusion criteria: Chronic low back pain (> 3 months); age between 25 and 60 years; absence of severe neurological pathology.

Rehabilitation program: Patients underwent a 16-week Pilates-based training program, conducted 2–3 times weekly under the supervision of a qualified therapist. The program included exercises for core stabilization, breathing control, strengthening of deep musculature, and improvement of postural control.

Results and discussion

The results of this study show a significant reduction in pain intensity and improvement in functional capacity following the 16-week Pilates-based rehabilitation program. The reduction in Visual Analog Scale (VAS) scores indicates a clinically significant decrease in pain. This effect can be explained by the activation of deep core stabilizing muscles, including the *m. transversus abdominis*, *m. multifidus*, and pelvic floor muscles, which play a key role in lumbar segment stability (Table 1).

Table 1. Results before and after the Pilates-based rehabilitation program

Parameter	***Pre-therapy***	***Post-therapy***	***P-value***
VAS (Pain Score)	*6.8 ± 1.2*	*3.1 ± 1.0*	*< 0.01*
Functional Stability Test (sec.)	*18.5 ± 4.2*	*31.7 ± 5.1*	*< 0.01*
Core Control (Score)	*2.4 ± 0.6*	*4.1 ± 0.7*	*< 0.01*

P < 0.05 is considered statistically significant.

In addition to the reduction in pain intensity, a significant improvement in functional stability and core control was observed (Fig. 1). The applied Pilates exercises are characterized by controlled movements, coordinated breathing, and a focus on correct postural alignment, leading to the optimization of neuromuscular control and enhanced motor coordination.

The obtained results confirm the positive effect of Pilates exercises on lumbar segment stabilization and movement control. This improvement can be attributed to the activation of deep stabilizing muscles and the optimization of postural control mechanisms.

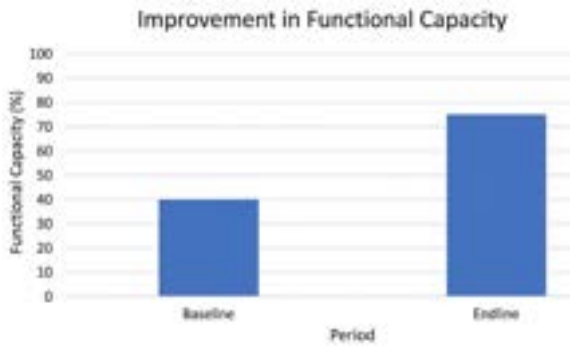

Figure 1. Functional stability and core control

The results of the present study are consistent with data from numerous randomized controlled trials, which demonstrate that Pilates-based interventions lead to substantial improvements in functional capacity and a reduction in the degree of disability in patients with chronic low back pain (LBP). A systematic review by Wells et al. (2012, 2014) shows that Pilates exercises lead to a significant reduction in pain and improvement in functional status among patients with chronic LBP [9, 10]. Similar results were reported by Ferreira et al. (2006), who found that Pilates programs are more effective than minimal intervention and comparable to other forms of therapeutic exercise [11]. Patients participating in Pilates programs demonstrate a statistically significant reduction in pain intensity and improvement in quality of life compared to control groups receiving standard care or minimal physical activity (Lim et al., 2024, 2011) [7, 12].

Recent meta-analyses have established that the effect on pain intensity and functional disability is statistically significant and, in many cases, superior to minimal or non-specific interventions [6, 13]. According to SUCRA analysis, the most effective interventions for reducing disability are Pilates, strength training, and abdominal exercises. Pilates had the highest probability of reducing pain (93%) and disability (98%) [14]. From a biomechanical perspective, the Pilates method supports the restoration of normal function of the muscular “core”, which reduces the load on the passive structures of the spine. Improved core control leads to better stabilization of the lumbar segment and a reduction in microtrauma that may sustain chronic pain [15]. There is low to moderate certainty that exercise therapy and leisure-time physiotherapy are beneficial for pain relief and the prevention of recurrence [16].

Conclusion

Pilates-based rehabilitation programs represent an effective and safe method for treating patients with chronic non-specific low back pain. Integrating them into standard rehabilitation protocols can improve functional independence and quality of life for patients. Despite the positive results, the present study has certain limitations. The primary limitation is the relatively small sample size and the absence of a control group. Future research with a larger number of participants and randomized controlled designs could provide more conclusive evidence

regarding the long-term effectiveness of Pilates-based rehabilitation programs.

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VI. Dental Medicine Session

Study of the level of awareness among dental students regarding the clinical and epidemiological characteristics of herpes zoster

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Herpes zoster is due to reactivation of the varicella-zoster virus in people who have already had chickenpox, but patients with herpes zoster can be a source of chickenpox for those who have not had it. The transmission mechanism is airborne and is realized upon contact with exudate from the vesicles or inhalation of aerosolized particles, and an aerosol is formed during almost every dental procedure. The **aim** is to assess the awareness of dental students about the clinic, mechanisms of transmission, and prevention of spread of the infection in their practice with patients. **Materials and methods:** A survey was conducted among 134 dental students. The anonymous survey reached the students via QR code and a direct group survey in paper form. The period of implementation is April–October 2025. **Results:** The cause of Herpes zoster, according to only 36.6% (n = 49) is Varicella-zoster virus. The rash is unilateral along the course of a peripheral nerve according to 45.5% (n = 61). Mechanism of transmission according to 36.6% (n = 49) is through direct contact with the lesions, but only 11.9% (n = 16) – by airborne droplets, through direct contact with the lesions and through contaminated instruments. Measures to prevent the spread are correctly indicated by 30.6% (n = 41) of personal protective equipment, disinfection, protective coverings, and delaying treatment. **Conclusion:** The risk of cross-transmission during oral cavity manipulations is real and requires knowledge of the clinic and anti-epidemic measures for reliable prevention of cross-infections in the dental office.
Keywords: *herpes zoster, cross-infections, dental practice*

Introduction

Herpes zoster is an acute infectious disease caused by Varicella-zoster virus (VZV) – a DNA virus of the Herpesviridae family. Herpes zoster itself is not transmitted from person to person, but is due to reactivation of the varicella-zoster virus (VZV) in people who have already had chickenpox.It is usually clinically presented as a painful herpetic rash located unilaterally along the course of the affected peripheral nerve. Oral manifestation of Herpes zoster occurs when the Varicella zoster virus reactivates in the branches of the trigeminal nerve, especially n. maxillaris or n. mandibularis. Burning, stinging, tingling, or pain is observed in the affected part of the face or oral cavity. Rash and lesions in the oral cavity are represented by vesicles, which subsequently form ulcers on the palate, tongue, gums, or the inner surface of the cheek, only on one side of the midline of the face. The transmission mechanism is mainly airborne and is realized by direct contact with exudate from the vesicles or inhalation of aerosolized particles from the vesicles until they dry. Given the specificity of dental manipulations, the formation of an aerosol in almost every treatment and prophylactic procedure, and direct contact with the patient at work, the dental office is a risky place for the transmission of this infection. This requires dentists, incl. and students of dental medicine, to be familiar with the main clinical manifestations and anti-epidemic measures that should be taken when treating a patient with this infection [1, 2, 3, 4, 5, 6, 7].

Aim

to assess the level of awareness of dental students about the clinic, the mechanisms of transmission, and the prevention of the

spread of herpes zoster in their practice with patients during clinical exercises.

Materials and methods

A survey was conducted among 134 dental students of the fourth year of Bulgarian-language training, who already had clinical practice with patients. Data was collected through an anonymous questionnaire prepared for the study, which reached the students through professionally oriented websites, as well as through a QR code provided for scanning and through a direct group survey in paper form. Participants previously filled out an informed consent form, in which they expressed their desire to participate in the survey. The period of conduct is April-October 2025. Statistical processing of the data was carried out using the statistical package SPSS Statistics, version 23.0.

Results

All students 100% (n = 134) are in the fourth year of their studies and are 22–23 years old. According to 63.4% (n = 85), the causative agent of herpes zoster is the herpes simplex virus, whereas 36.6% (n = 49) report the varicella-zoster virus. No one indicated cytomegalovirus and "I am not sure" as possible answers. The rash is unilateral along the course of a peripheral nerve in 45.53%% (n = 61), but in 35.07% (n = 47) it is always bilateral, which indicates a complete lack of understanding of the clinical course of the infection. 14.93% (n = 20) give the optimal answer, namely a unilateral rash along the course of a peripheral nerve, preceded by pain/paresthesia. 4.47% (n = 6) are not sure which is the correct answer. **Fig. 1.**

Regarding the mechanism of transmission, we report: According to 36.6% (n = 49), it is through direct contact with the lesions; for 20.1% (n = 27) – through contaminated instruments/surfaces; for 11.9% (n = 16) – through airborne droplets, through direct contact with the lesions, as well as through contaminated instruments; according to 9.7% (n = 13) – through contaminated instruments and in contact with blood; 4.5% (n = 6) – through contaminated instruments and through airborne droplets; 9.0% (n = 12) allow all mechanisms to be possible without airborne transmission; 8.2% (n = 11) are not sure of the correct answer. The following complications were indicated as possible by the respondents: Prolonged postherpetic neuralgia – 29.85% (n = 40); Secondary bacterial infection of the lesions – 8.9% (n = 12). In immunosuppressed patients, spread with rashes outside the innervated area is possible – this is a correct answer, but it is indicated only by 5.2% (n = 7). All answers are correct for 12.6% (n = 17); Neuralgia, together with secondary bacterial infection occurs according to 5.2% (n = 7); Neuralgia, together with the spread of rashes outside the innervated area in immunocompromised patients, is observed according to 11.2% (n = 15); Dissemination together with secondary infection is indicated by 9.7% (n = 13), and 17.16% (n = 23) are not sure. Differential diagnosis of pain characteristic of postherpetic neuralgia, according to 40.29% (n = 54) is made with periodontitis, and this answer is correct. However, the majority gave other answers: 52.23% (n = 70) believe that this is pulpitis, 3.73% (n = 5) – stomatitis, and 3.73% (n = 5) are not sure. According to 94.77% (n = 127) the dental office is a risky place for transmission and spread of the virus, and 5.2% (n = 7) do not assess it as such. Effective measures to prevent the spread are shown in **Fig. 2**. The most optimal is the implementation of all measures together.

Despite their incomplete knowledge, not all students want to participate in additional training on the topic "Oral manifestation of infectious diseases", only 85.82% (n = 115). 5.9% (n = 8) do not consider this necessary.

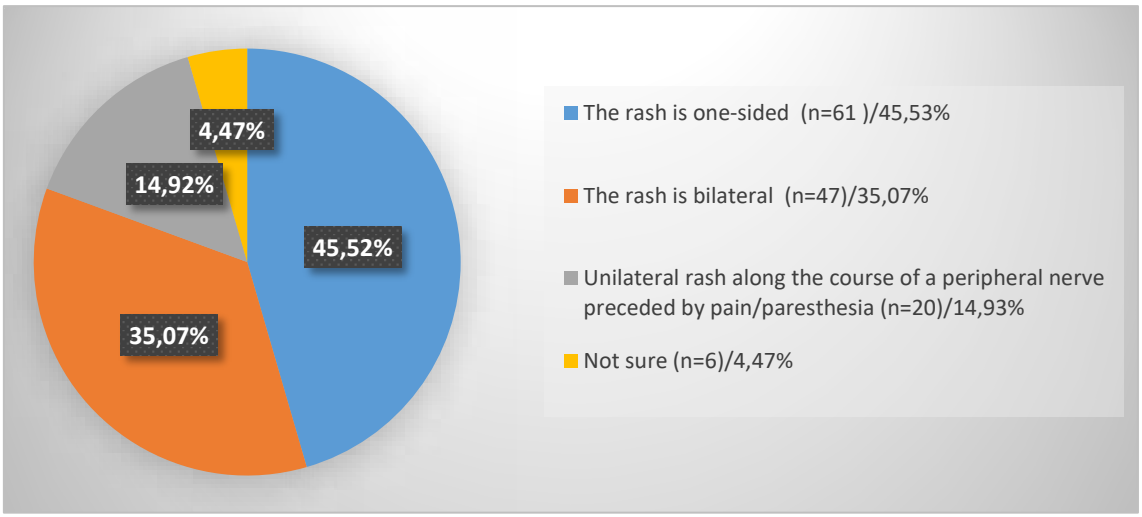

Figure 1. Distribution of responses regarding clinical presentation

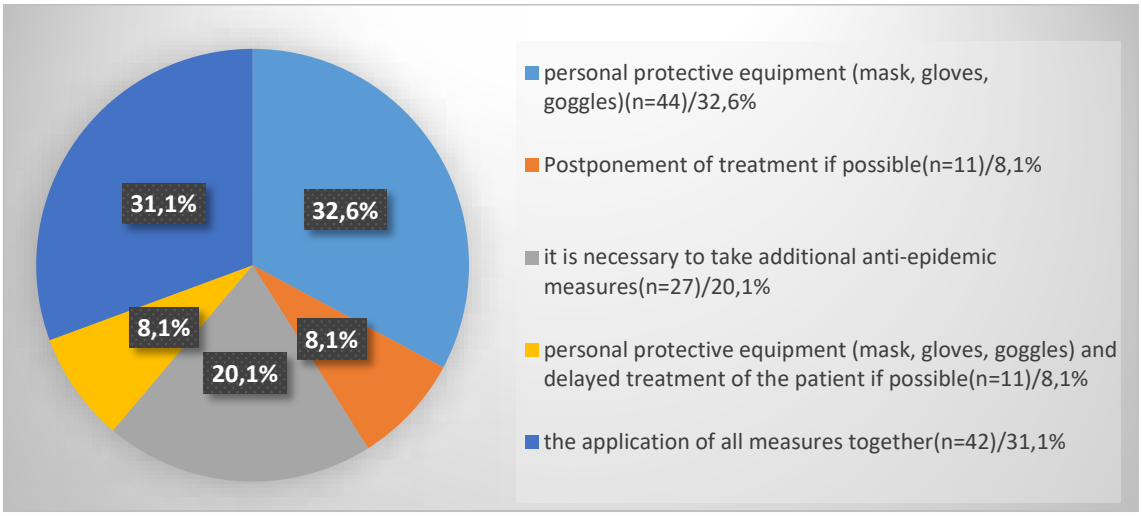

Figure 2. Effective measures to prevent the spread of Herpes Zoster

Discussion

Dentists, including dental students, play an important role in the identification and treatment of oral ulcers in herpes zoster virus infection. Knowing the oral manifestations of herpes zoster, they could diagnose it in its early stages. Authors describe a wide range of oral symptoms – lesions on the soft palate, palatal schwannoma, vascular anomalies, lipoma, oral fibroma, painful vesicles that appear in the area innervated by the corresponding nerve, together with blisters and ulcerations in the oral cavity, as well as difficulty swallowing [8]. Oral manifestations of herpes zoster are observed when the mandibular and maxillary branches of the trigeminal nerve are affected by the virus. The trigeminal nerve is affected in about 20% of cases. Early diagnosis plays a very important role in limiting the spread of the infection [9]. According to 27% (n = 62), the rash is unilaterally located along the course of a peripheral nerve, preceded by pain/paresthesia, and this is an optimal response. According to 21.7% (n = 50), the rash is always bilateral, which suggests an automatic response. In immunocompetent patients, the virus can cause large local lesions or disseminated infection. Postherpetic neuralgia is a potential consequence of Herpes Zoster, and

can sometimes persist for months to years after the lesions have healed [10–11]. As a complication after Herpes Zoster, prolonged postherpetic neuralgia was mentioned by 17.4% (n = 40), and dissemination with rashes outside the innervated area in immunosuppressed patients and neuralgia is possible according to 10.9% (n = 25), which indicates an insufficiently thorough knowledge of the possible complications.

Conclusion

It is important for dental students and doctors to be able to recognize the condition by unilateral lesions and preceding pain and to make a differential diagnosis with other dental infections (periodontitis); In active lesions, it is advisable to postpone scheduled dental procedures because the patient is infected. Strict implementation and always in their entirety of standard and additional precautions; It is important to know the ways of transmission of the infection – VZV can be transmitted through contact with the lesions or aerosols; Conducting an epidemiologically focused history. Factors increasing the risk in dental practice: Patient with active lesions on the face, lips, or intraorally; Aerosol-generating procedures (using turbines, ultrasound, polishing); Insufficient disinfection and/or lack of barrier protective equipment; Unvaccinated personnel who have not had Varicella.

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Fourth year students` perception about artificial intelligence in dental medicine education

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Introduction: Artificial intelligence (AI) is expanding in all areas of modern life, prompting a gradual shift in the educational paradigm of dental medicine. Data addressing students' experiences and perceptions remain limited. **Aim:** The aim of this study was to assess fourth year dental students' experiences, perceptions, and expectations regarding artificial intelligence in dental medicine education. **Materials and methods:** An anonymous digital questionnaire survey (Microsoft Forms) was conducted in January 2026 among fourth year dental students at Medical University of Plovdiv. It consisted of 20 questions to collect demographical data and opinions concerning AI in dental education. Statistical analysis was performed. **Results and discussion:** A total of 43 international students completed the survey, predominantly female (65%), most aged 20–25 years (93%). Fourty percent of the respondents found the use of AI most helpful in understanding and diagnosing dental conditions. Moderately confident in decisions that incorporate AI assistance were 72%. Most respondents (79%) expressed the need for their educational institution to provide AI training. Frequency of use was significantly associated with perceived educational impact ($p < 0.05$), and female students reported significantly higher usage than male students ($p < 0.05$). These findings indicated engagement with AI, while highlighting persistent gaps in regulatory awareness. **Conclusion:** Fourth year dental students demonstrated continued reliance on AI and strong support for structured education in this field, highlighting the need for curriculum integration.

Keywords: *artificial intelligence; dental education; questionnaire survey; student perception*

Introduction

Artificial intelligence (AI) is expanding in all areas of modern life, prompting a gradual shift in the educational paradigm of medicine [1–3].

In dentistry, AI is increasingly being applied in diagnostics, treatment planning, and educational support, enhancing both clinical decision-making and the learning process of students [4]. As these technologies continue to evolve, they are expected to play a more significant role in shaping the future of dental practice and education. An increased understanding of how dental students perceive AI can be essential for guiding the effective integration of these technologies into dental curricula in the future. Students' attitudes, confidence, and expectations toward AI will directly influence its adoption and appropriate use in clinical settings [5, 6]. Data addressing students' experiences and perceptions remain limited.

Aim

The aim of this study was to assess fourth year dental students' experiences, perceptions, and expectations regarding artificial intelligence in dental medicine education.

Material and Methods

An anonymous digital questionnaire survey in Microsoft Forms was conducted in January 2026 among fourth year dental students at the Faculty of Dental Medicine, Medical University of Plovdiv. The study was approved by the institutional Scientific Ethics Committee of Medical University of Plovdiv (Plovdiv, Bulgaria), #P-KHE-40/14.04.2026 following the 1964 Helsinki declaration and its later amendments.

The questions in the survey were divided in two main parts: the educational and the ethical opinions of the dental students. The questionnaire consisted of 20 questions to collect demographical data, opinions

concerning the use of AI by students for their medical education and the potential of it to facilitate and improve professional dental management and patient treatment. Statistical analysis was performed, using SPSS v26 (Chi-square, Spearman correlations, Mann–Whitney U).

Results

A total of 43 fourth-year dental students participated in the study. The majority of participants were undergraduate dental students (95%), with a small proportion of graduate students (5%). Most respondents had been studying dental medicine for 2–4 years (93%), while 7% reported more than 4 years of education. Participants were predominantly aged between 20 and 25 years (93%), with a minority over 25 years (7%). The sample consisted mainly of female students (65%), followed by male students (33%), and 2% who preferred not to disclose their gender. The majority of participants originated from the United Kingdom, followed by smaller groups from Germany, Italy, and Greece.

Use of artificial intelligence in education. AI-based tools were widely used among participants. More than half of the students (58%) reported frequent use, while 35% used AI occasionally and 7% reported rare or no use. AI was most commonly utilized for understanding and diagnosing dental conditions (40%), followed by case-based learning (28%) and learning new techniques and procedures (19%). Less frequent applications included treatment planning and patient data management (2% each).

Perceived benefits of AI. Participants identified faster and more accurate diagnostics (40%) as the main benefit of AI in dental education and practice. Other reported advantages included improved treatment planning (23%) and enhanced patient data management (19%), while 19% indicated additional benefits.

Perceived limitations and challenges. The most frequently reported limitations were limited accuracy (37%) and ethical or legal concerns (35%). Additional concerns included limited potential for personalized treatment (19%) and other factors (9%).

Confidence in AI-assisted decision-making. Most participants reported moderate confidence in AI-assisted clinical decisions (72%), while 16% indicated high confidence and 12% reported low confidence.

Perceptions of AI impact on dentistry. Nearly half of the respondents (49%) anticipated a significant impact of AI on future dental practice, while 42% expected a moderate impact and 9% predicted a minor influence. Similarly, 63% of participants believed that AI would have a significant impact on dental education and training, with 35% indicating a moderate impact.

Ethical considerations. Regarding ethical aspects, 51% of participants expressed a positive attitude toward AI use in dentistry, while 47% were undecided and 2% reported negative opinions. Despite this, awareness of regulatory frameworks was low, with 58% of participants reporting no familiarity, 37% limited familiarity, and only 5% indicating a high level of awareness.

Educational needs and institutional support. A substantial majority of students (79%) reported that their institution does not provide sufficient training on AI in dentistry, while 16% were uncertain and only 5% considered the training adequate.

AI tools and future expectations. The most commonly reported AI tool used by students was ChatGPT, primarily for simplifying complex concepts and answering academic questions. Other tools included Google Gemini, Grammarly, and NotebookLM. However, a notable proportion of

students reported not using any specific AI tools. Students' expectations regarding the future role of AI were generally positive, with emphasis on its function as a supportive clinical tool, particularly in diagnostics and treatment planning.

Qualitative insights. Qualitative responses highlighted the role of AI as a learning aid, particularly for understanding complex concepts. Students emphasized the importance of critical evaluation of AI-generated information and expressed a strong need for institutional AI tools based on validated scientific data and real clinical scenarios.

The statistical analyses (Chi-square, Spearman correlations, Mann–Whitney U) showed that frequency of use was significantly associated with perceived educational impact ($p < 0.05$), and female students reported significantly higher usage than male students ($p < 0.05$). These findings indicated engagement with AI, while highlighting persistent gaps in regulatory awareness.

Discussion

The results show that AI is widely used among fourth-year dental students, particularly as a tool to support studying and learning. Most students reported frequent use of AI, mainly for simplifying complex concepts and improving understanding of dental conditions, as well as for case-based learning. This highlights the important role of AI in enhancing academic performance and learning efficiency [7]. At the same time, most students were only moderately confident in decisions supported by AI, indicating that these tools are helpful but not fully trusted for independent clinical use. Students also emphasized the need to use AI critically and not rely completely on its outputs [4, 5].

AI was generally perceived as having a significant or moderate impact on the future of dentistry, mainly as a supportive tool rather than a replacement for the dentist. However, key concerns were identified, particularly related to limited accuracy and ethical or legal issues [8, 9].

Despite the high level of use, most students reported insufficient training provided by their institution and low awareness of AI regulations. This highlights a gap between practical use and formal education, indicating the need for better integration of AI into the dental curriculum with proper guidance and training [10].

Conclusion

Fourth year dental students demonstrated continued reliance on AI and strong support for structured education in this field, highlighting the need for curriculum integration. Introducing additional educational programs and structured AI training across different dental fields might improve understanding of AI capabilities and limitations as well as increased awareness of ethical and legal responsibilities.

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Crowns in paediatric dentistry

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Introduction: Crowns in paediatric dentistry are prefabricated crown forms adapted to individual teeth and cemented with a biocompatible luting agent, providing full coverage restoration for primary dentition of severely damaged teeth. **Aim:** To systematically review the contemporary scientific literature concerning the basic types of paediatric crowns used in primary teeth. **Materials and methods:** The search strategy involved the most popular international scientific databases such as PubMed, Scopus, Web of Science, and Google Scholar. Only articles in English were included in the analysis. The research focuses on the following types of scientific articles: original articles, systematic reviews, meta-analyses, case reports, handbooks, and guidelines. To provide contemporary and modern knowledge on the topic discussed, only scientific literature from the last 5 years was analyzed. **Results and discussion:** The expository analysis summarizes the characteristics, indications, and contraindications for the use of paediatric crowns, as well as the potential complications. A critical evaluation of the advantages and disadvantages of the described variety of crowns has been made. The article provides insight into the clinical protocol of different crowns for the right therapeutic approach. **Conclusion:** There is a need to build awareness among dentists associated with the use of paediatric crowns in primary dentition. Future studies should also seek the long-term effects of the new versions of crowns for primary teeth, which will be interesting as the profession begins adopting them in everyday dental practice.

Keywords: *crowns, primary teeth, paediatric dentistry*

Introduction

Oral health during the primary and mixed dentition periods is often neglected by parents and some dentists. In fact, this is a crucial period of growth and development, important for the overall well-being of the child. The treatment of severely decayed primary teeth is a challenge for the pediatric dentist and is determined by the patient's behavior, age of the child, preservation of hard dental tissues, and parental satisfaction [1, 2].

Crowns in pediatric dentistry are prefabricated crown forms adapted to individual teeth and cemented with a biocompatible luting agent, providing full coverage restoration for primary dentition of severely damaged teeth [1, 3].

Aim

The aim of this article is to systematically review the contemporary scientific literature concerning the basic types of pediatric crowns used in primary teeth.

Material and methods

The search strategy involved the most popular international scientific databases such as PubMed, Scopus, Web of Science, and Google Scholar. Only articles in English were included in the analysis. The research focuses on the following types of scientific articles: original articles, systematic reviews, meta-analyses, case reports, handbooks, and guidelines. To provide contemporary knowledge on the topic, only scientific literature from the last 5 years was analyzed.

Results and discussion

The expository analysis summarizes their characteristics, indications, and contraindications for use, as well as the potential complications. A critical evaluation of the advantages and disadvantages of the described variety of crowns has been made.

The pediatric crowns used in primary dentition are divided into several types based on the material and clinical outcomes (Figure 1) [1–3]. It should be noted that each type of crown has subtypes based on the composition, esthetics, morphology, occlusal anatomy, characteristics, etc. (Table 1) [2, 4]. To facilitate the choice of the most appropriate

crown type, a broad range of factors such as indications and contraindications, aesthetics, price, and additional requirements are considered [1–4].

Preformed metal crowns (PMCs) are divided into several groups based on composition, aesthetics, and morphology. The most widely used crowns in pediatric dentistry are traditional Stainless steel crowns (SSCs). Despite a wide variety of pediatric crowns available in modern practice, SSCs are still commonly used and considered a successful treatment modality [2–4]. Numerous studies reported that SSCs had superior clinical performance as restorative constructions for posterior primary teeth [2–6].

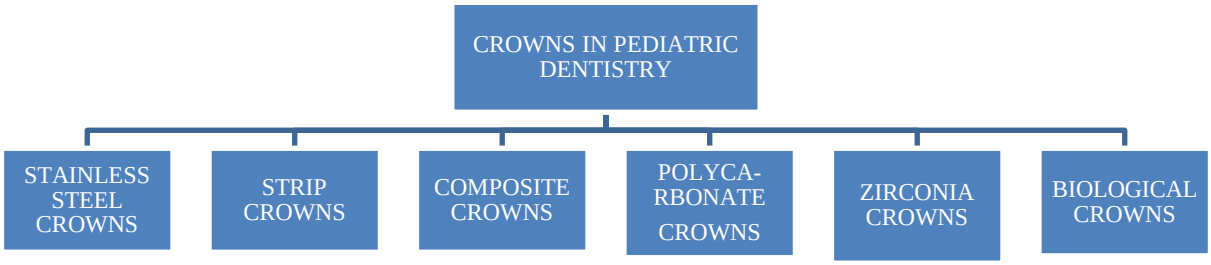

Figure 1. Types of crowns in pediatric dentistry

Table 1. Subtypes of PC

SUBTYPES OF SSCs	
BASED ON THE TYPE AND AESTHETICS	Stainless Steel crown
	Pre-veneered SSC
	Open-faced SSC
	Pedo pearl
BASED ON THE COMPOSITION	Stainless Steel crown
	Nickel-Base crowns
	Tin-base crowns
	Aluminum-base crowns
BASED ON MORPHOLOGY (FORM AND CONTOUR)	untrimmed (requires much time for adjustment)
	pre-trimmed (require additional contouring)
	pre-contoured (most widely used – duplicates the shape of the molars, provides good retention)
SUBTYPES OF THE PREVENEREERED SSCs	
BASED ON THE OCCLUSAL ANATOMY	occlusally small
	smallest and least occlusally carved
	compact occlusal anatomy
	shallow occlusal anatomy
	ideal occlusal anatomy
SUBTYPES OF COMPOSITE CROWNS	
BASED ON THE COMPOSITION	Pedo pearl
	New millennium crowns
	Pedo Jacket Crown
	Bioflx crowns

Open-faced SSCs are used in the anterior section of the dental arch. The procedure includes adapting the proper SSC, followed by cutting away the cosmetically prominent aspect of the crown, removal of part of the luting agent to leave retentive undercuts, and filling the void with bonded resin composite. In this way, the result is a combination of the strengths of SSCs and the improved appearance of affected teeth [3–6].

Pre-Veneered SSCs are similar to open-faced SSCs, as they combine the mechanical properties of SSCs with an aesthetic appearance. However, the aesthetic part is a composite or a thermoplastic resin chemically or mechanically bonded to the crown [4–6].

Pedo Pearl is considered a version of pre-veneered crowns. The base of these crowns is made of aluminum covered with epoxy paint for aesthetic appearance. In addition, composite resin can be applied as a coverage of the crown [2, 4]. Like SCCs, adaptation requires cutting and crimping. The authors reported an extremely soft structure of the crown, making them unreliable constructions for restoration till exfoliation [4, 6].

Polycarbonate crowns are made of thermoplastic resin. First, they are widely used to restore severely damaged anterior primary teeth due to their aesthetic appearance. Later, the insufficient material properties, thin structure, and great flexibility resulted in low resistance to high abrasive forces, crown fracture, or premature crown wear [6].

Strip crowns (SCs) are transparent plastic forms used to restore severely damaged anterior primary teeth in a simple, cost-effective, and satisfactory way to the parent [4–6]. This is the first choice of dental practitioners due to the high aesthetic appearance and easy repair in case of fracture or wear and tear [2]. However, the technique is sensitive as it requires perfect isolation of the operatory field. Resin-based composite SCs offer a balance of aesthetics and function [5].

Pedo jacket crowns are similar to SCs. The main differences are the material they are made of – tooth-colored copolyester – and the clinical protocol – the operator does not remove them after light-curing, (Pedo jacket crown remains on the tooth) [5–7]. The fixation is performed by filling the crown with resin composite and bonding it to the tooth. Due to the temperature generated during the rotation of the high-speed finishing bur, trimming and contouring are not possible [4].

New Millennium crowns are also similar to SCs. The main difference is the material they are made of – laboratory-improved composite resin. Like the SCs and Pedo jacket crowns, the New Millennium crown form is filled with resin composite and bonded to the tooth [2, 6]. On one hand, New Millennium crowns are aesthetic and can be trimmed with a high-speed bur. On the other hand, they are fragile and require a dry operatory field. These disadvantages are the reason for the rejection of their use [7].

Zirconia pediatric crowns (ZPCs) demonstrate perfect aesthetic colour, biocompatibility, and high wear-resistance. In accordance with parental satisfaction with their tolerance to periodontal tissues, fracture resistance, long-lasting restoration till exfoliation, currently, ZCs are widely used for severely destroyed anterior primary teeth [5–7].

Bioflx crowns (BFCs) are the first pediatric crowns that are flexible, durable, and esthetic in appearance. They are made of biocompatible hybrid resin polymer [8]. When compared to the traditional SSCs, they are saving chair time and requiring fewer adjustments. Researchers report easy preparation (between ZCs and a traditional SSC preparation) and easy adaptation. The innovative

properties of the BFCs are the active fit for easy placement and self-adaptive technology – forming a "dimple" in areas of high occlusion (instead of wearing). The wear resistance of BFCs is similar to or better than that of traditional SSCs. They have a radiopacity of 1 mm that permits evaluation of the crown margins and pulp capping materials. No visible discoloration after more than 1 year intraorally is found. For enhanced retention with cementum, a sandblasted intaglio surface is provided [8, 9].

Indications and contraindications

The indications for the use of pediatric crowns in primary teeth are tooth age – at least 2 years up to exfoliation, incisional edge affected by caries, early childhood caries, extensive decay of primary teeth, children at high risk of oral diseases, treatment under general anesthesia, restoration of dysplasia, after endodontic treatment, restoration of a fracture, lack of a permanent tooth germ, no possibility for operatory field isolation, extensive physiological or paraphysiological abrasion, abutment for a space maintainer, non-restorable teeth with a restoration, severe bruxism [2, 4, 7, 9].

Contraindications for the use of pediatric crowns are divided into general contraindications and specific contraindications (Table 2) [4, 7, 9].

General contraindications for use are restorable teeth with a conventional filling, lack of sufficiently hard tissues for retention, periodontal disease, resorption of more than ½ of the root, increased mobility due to exfoliation, a primary tooth near its exfoliation time (up to 3–6 months), and uncooperative behaviour of the patient.

Pediatric crowns provide full crown coverage, promising a significantly improved alternative to conventional restorations. By protecting entire tooth, crowns are advocated for their superiority in prevention of dental caries as well as the management of the carious process and adjacent caries development in primary teeth [5, 6, 9].

Table 2. Specific contraindications for the use of pediatric crowns in primary dentition

SSCs	• patients with nickel allergies • extensive loss of hard dental tissue due to attrition • abrasion • dysplasia
OPEN-FACED SSCs	• patients with nickel allergies • fracture line below the gingival margin
POLY-CARBONATE CROWNS	• excessive tooth damage that is insufficient for retention • too small teeth in size • crowded anterior teeth • bruxism • excessive abrasion • overbite • deep impinging bite
STRIP CROWNS	• significant tooth tissue loss, preventing proper retention • deep overbite • periodontal disease
NEW MILLENIUM CROWNS	• difficulty in keeping the restoration area dry • extensive tooth damage that is insufficient for retention • periodontal disease • overbite • deep impinging bite

Table 3 provides a comprehensive comparison of advantages and disadvantages of pediatric crowns [9, 10]. This comparative analysis is designed to assist dentist in decision whether to restore the tooth with a restoration or use a pediatric crown.

Many researchers present potential complications when using different types of pediatric crowns [2, 6, 9, 10]. Registering them is important, since complications serve as outcome measures and indicators of quality of care. Crowns in pediatric dentistry are considered a safe and successful method for the restoration of damaged primary teeth. However, possible complications during the preparation and application of crowns are swallowing of the crown, aspiration of the crown, poor marginal adaptation, potential for microleakage, gingival inflammation, incorrect inclination after crown placement, interproximal threshold, and overcontouring of the crown [5, 7, 8, 10].

Advantages and disadvantages

Table 4 succinctly summarizes specific advantages and disadvantages of the most commonly used pediatric crowns in primary dentition, presenting a quick, reliable, and objective overview for dental professionals. This comparative analysis is designed to assist quick and effective choice for the most suitable crown type in the treatment of pediatric dental patients [2, 5, 7, 8].

Table 3. Advantages and disadvantages of pediatric crowns in primary dentition

ADVANTAGES	DISADVANTAGES
• prevention of premature tooth loss • prevention of malocclusion • prevention of psychological problems • protection of the residual tooth structure weakened after excessive caries removal • restoration of the chewing function • low risk of making errors during their cementation in case of gingival bleeding and lack of isolation of the surgical field application (for SSCs) • longevity is the same as that of an intact primary tooth • good long-term cost effectiveness • aesthetic • different sizes • require a dry operative field (for strip crowns)	• require removal of a large amount of hard dental tissues (for ZCs) • unsightly metallic appearance (for SSCs) • gingival hyperplasia • cannot be used in case of a partially erupted tooth • high price

Table 4. Specific advantages and disadvantages of the most commonly used pediatric crown in primary dentition

CROWN TYPE	ADVANTAGES	DISADVANTAGES
SSCs	• low price • fast modifiability and fit • anatomical shape • easy technique • low failure rate • durability	• no esthetics • it cannot be used when the tooth has only partially erupted • potential for microleakage • gingival inflammation
STRIP CROWNS	• easy to fix • thin proximal walls of the crown • available tools for adjustment in every dental office • minimal removal of hard dental tissues • excellent aesthetics • variety of sizes available	• sensitive technique • an adhesive system is required • fragile structure • possible discoloration of the crown • risk of incomplete polymerization • proper isolation is crucial • low durability
ZIRCONIA PEDIATRIC CROWNS	• aesthetics is not affected by moisture • high aesthetic • highest strength of all pediatric crowns	• require deep preparation that can lead to pulp involvement • cannot be crimped • no adjustment is possible • require good isolation • expensive • not flexible
BIOFLX CROWNS	• flexible • self-adaptable • durability • esthetic • active fit • pre-contoured and pre-crimped, time-saving	• adhesive and cohesive failures • chipping of the composite layer under excessive occlusal load

Conclusion

The article highlights that a variety of pediatric crowns used in primary dentition are described in scientific dental literature. However, there are a few types of crowns reported by pediatric dental practitioners as the best available treatment option. Compared to all contemporary restorative materials, pediatric crowns in primary dentition are integral to managing decay and are more durable than multisurface conventional restorations. There is a need to build awareness among dentists about indications and contraindications, advantages, and disadvantages. Future studies should also seek the long-term effects of new versions of crowns for primary teeth, which will be interesting as the profession begins adopting them in everyday dental practice.

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Study of the oral health of children actively playing sports, children football players

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Rational nutrition is crucial for optimal health and the prevention of adverse effects related to physical exertion. Unbalanced eating habits and insufficient intake of essential nutrients are observed in child football players. This can lead to fatigue, injuries, and impaired physical development. Poor oral health requires increased attention to dietary regimens. **Objective:** To study the eating habits of child football players and to assess the main risk factors for oral health, with a view to preventing adverse health consequences. **Materials and methods:** A survey was conducted among 400 child football players in age groups, from eight to seventeen years of age. A questionnaire was developed, aimed at their basic eating habits and knowledge of maintaining oral health. Results were compared with the registered carious lesions by groups. The period is May 2023 – March 2025. **Results:** children consume mainly carbohydrate food and suboptimal amounts of proteins and fats. Inadequate dietary regimens correspond to the registered carious lesions: in eight-year-olds, 10.0% have carious lesions with a diagnostic threshold d1, and 22.5% have lesions with a threshold d2. In nine-year-olds, d2 reaches 32.50%, and in ten-year-olds 42.5%. There are carious lesions in the permanent dentition as well, D1 in eight-year-olds is 2.0%, in nine-year-olds 27.5%, and in twelve-year-olds 30.0%. **Conclusion:** The study of dietary habits is important because they influence sports productivity and contribute to the proper growth and development of adolescents. The identified gaps in dietary regimens reveal risky dietary habits that can lead to health problems if not corrected in time.
Keywords: *oral health, eating habits, diagnostic threshold, oral hygiene index, prevention*

Introduction

Oral health is part of general health and, as such, should be of great interest to health professionals. WHO defines oral health as a state in which there is no pain in the oral cavity and maxillofacial area, neoplastic changes, congenital defects, periodontal diseases, carious and non-carious diseases, and other conditions affecting the oral cavity [1]. Modern dentistry is oriented towards the prevention of diseases in the oral area, categorically emphasizing the relationship between oral health and general health [2]. Sports dentistry is emerging as one of the newest and still not sufficiently popular disciplines in our country. Its main task is to prevent and treat orofacial injuries and changes of sports origin. The dentist involved in sports dentistry has the task of diagnosing, treating, and preventing the undesirable effects that occur in the orofacial area during sports. In sports, the challenge is to maximize the benefits of participation and limit injuries. Prevention and adequate preparation are key to minimizing negative and risk factors during active sports [3]. The low oral health and the presence of risk among this group are facts, and this requires the definition and preparation of a program for the prevention and prophylaxis of these conditions.

Aim

To study the level of oral hygiene by determining the oral hygiene index according to the Greene-Vermillion (OHI-S) in actively playing sports, child football players, and to determine guidelines for prophylaxis.

Materials and methods

A prospective study was conducted, which included a total of 400 child soccer players divided into 10 age groups, from eight to seventeen years old. The oral hygiene index according to Greene Vermillion (OHI-S) was used to determine the oral hygiene status of the children. The data were statistically processed with SPSS v.19. For the study, a questionnaire was developed, aimed at the basic eating habits of the children, as well as knowledge about

maintaining oral health. The parents of the children previously filled out an informed consent form, in which they stated their desire to participate in the study. The study period is November 2025 – March 2026.

Results

The distribution of the OHI-S grades established in the study (first grade – coverage of the tooth surface with plaque – up to 1/3; second grade – coverage of the tooth surface with plaque – up to 2/3, and third grade – over 2/3 to the entire tooth surface, in the different groups of child footballers, is presented in **Figure 1.**

The figure shows that among the eight-year-old group we obtained the following distribution of results: the highest is the relative share with plaque index 0–1, (52.50%), which we actually accept as a satisfactory result. 35.00% is the relative share of children with grade 1.1–2, and only 12.50% is the relative share of children with grade 2.1–3, which is the most unfavorable result. It is striking that in the group of nine-year-old children, the distribution is approximately even. For 0–1, the relative share is 30.00%, for 1.1–2, the value is 37.50%, and 32.50% is the relative share at grade 2.1–3. Only in 1/3 is OHI-S 1.1-2 registered, which we consider to be a poor prognostic sign for poor oral health. The group of ten-year-old football players has the highest relative share at grade 1.1–2 (45.00%). At grade 0–1 the relative share is 40.00%, 15.00% is the relative share of the most severe degree. In eleven-year-old children the relative shares are equally distributed across the different degrees of severity, as in eight-year-old football players. Twelve-year-olds have the highest relative share at the mildest degree (55.00%). 15.00% is the share of children with grade 1.1–2 and the most severe grade 2.1–3 the relative share is 30.00%. In thirteen-year-old children, the highest relative share is registered in the medium severity grade – 52.50%, 35.00% is the relative share in children with the mildest grade 0–1, with the lowest relative share being established in the most severe grade (12.50%). In fourteen-year-old children, only data in the mild and moderate grades are registered. The relative share of the mild grade (0–1) is 80.00%, of the moderate 20.00%. In fifteen-year-old football players, the relative shares for the mild and severe grades are the same – 22.50%, the value of the relative share in the moderate grade is 50.00%. In sixteen-year-old children the highest relative share is in the moderate grade 45.00%, followed by a 40.00% relative share of the mild degree, with the lowest relative share being registered at the most severe degree (15.00%). In seventeen-year-olds, the relative shares of the moderate and severe degree are equal (42.50%). The relative share of the mild degree is 15.00%. The fact that among this group we find the most unfavorable OHI-S values is probably due to the consolidation of harmful habits, the excessive use of carbonated and energy drinks and the consumption of energy bars, which increases with age.

These suboptimal results are explained by tracking the eating habits of the studied groups of children.

Figure 2 shows the frequency of meals in one day.

It can be argued that as children age, the majority of them prefer increased food intake, which is expected and consistent with the increased energy expenditure. Adequate food intake provides energy both for the performance of training tasks and for the growth and development of children, which leads to full performance during training and competitions.

Figure 3 shows preferences for a specific type of food.

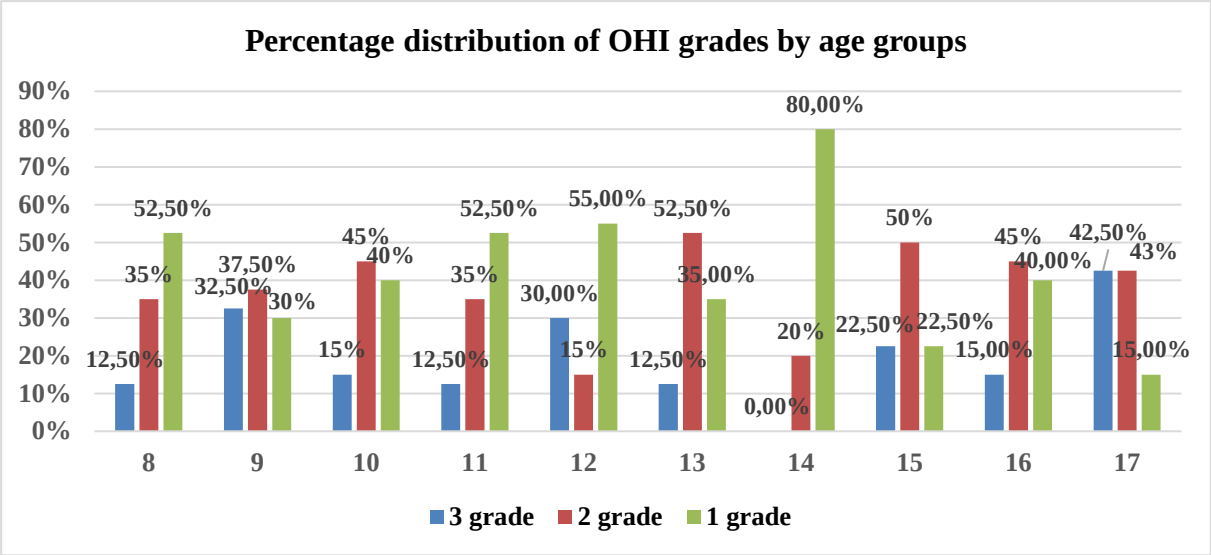

Figure 1. Distribution of the Greene Vermillion Oral Hygiene Index (OHI-S) grades by age groups

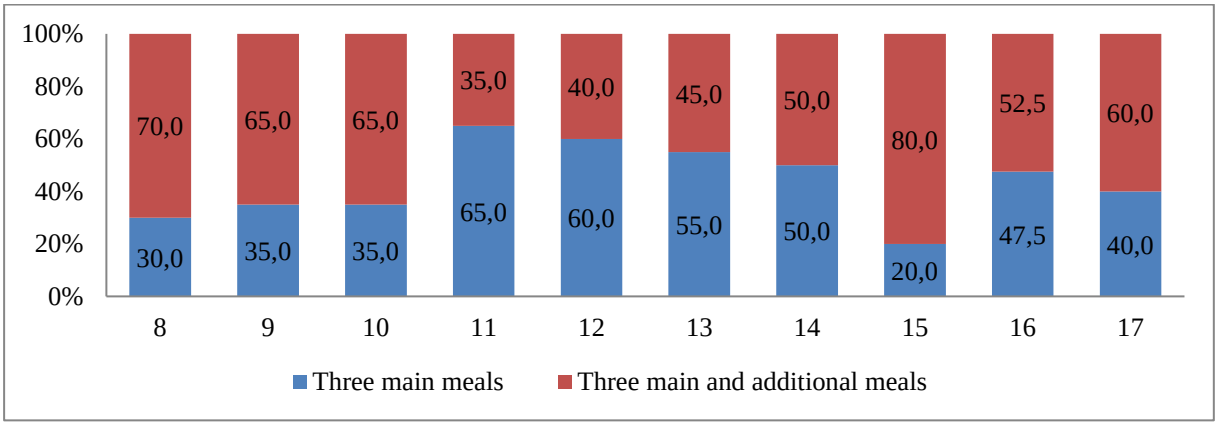

Figure 2. Frequency of meals per day

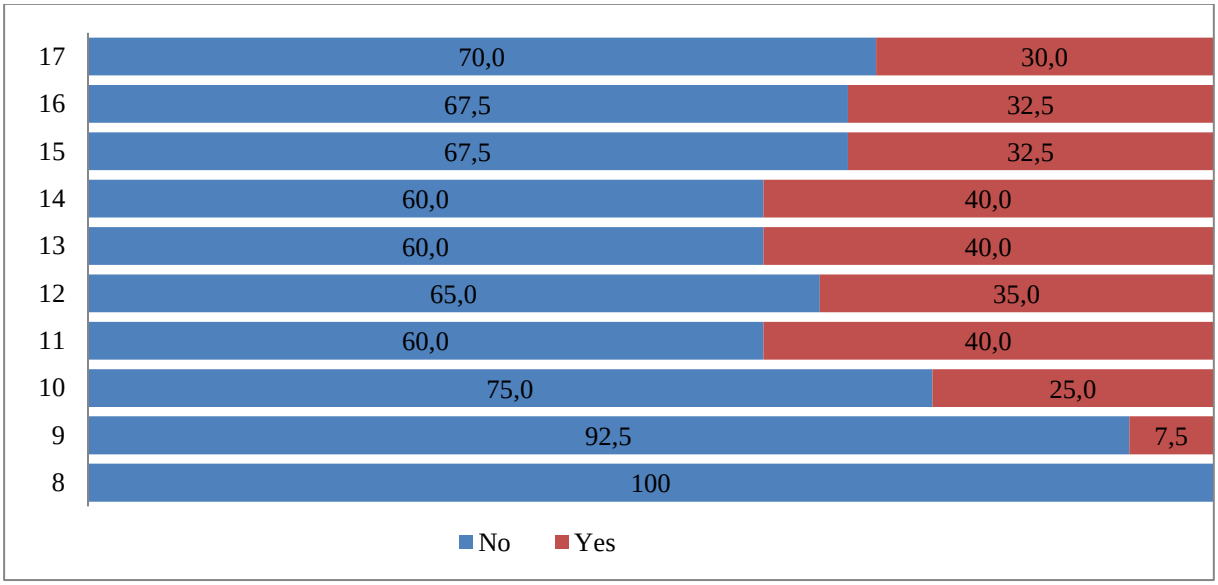

Figure 3. Diet and food restriction.

Child athletes prefer carbohydrate food, which is consistent with the increased energy expenditure. The energy spent during training can be compensated for most quickly by consuming foods with an increased carbohydrate content. It is evident that the majority prefer carbohydrate food (61.00%), and the remaining 37.50% and 1.50% protein and food rich in fat. For a specific explanation of whether these predominant carbohydrate foods are low-molecular, which have a proven cariogenic effect, we asked about the consumption of sweetened foods. **(Figure 4)**

Specifying the type of carbohydrate food, or more precisely, whether these foods are cariogenic, is important for identifying a possible risk indicator and, accordingly, for changing it. There is a clear prevalence of the percentage of children who consume sweet foods (84.5%). Only in the twelve-year-olds is there a closeness in the percentages, unlike the other groups, where there is a significant difference in favor of those children who consume sweet foods. The high percentage of children who consume such foods is a fact that speaks of the risk to oral health. It is known that many children, not only football players, consume carbohydrate foods, but the diagnosis of a change in saliva parameters can, in itself, potentiate the negative effect of these foods, which increases the risk of impaired oral health. In addition to the intake of carbohydrates in the form of food, the intake of sweet liquids is also important. A large part of the drinks consumed by children contain low-molecular carbohydrates. These are the various types of natural and fresh juices, carbonated drinks, and the now widely available energy drinks. We studied what type of liquids children prefer, and they were able to give more than one answer, as shown in **Figure 5.**

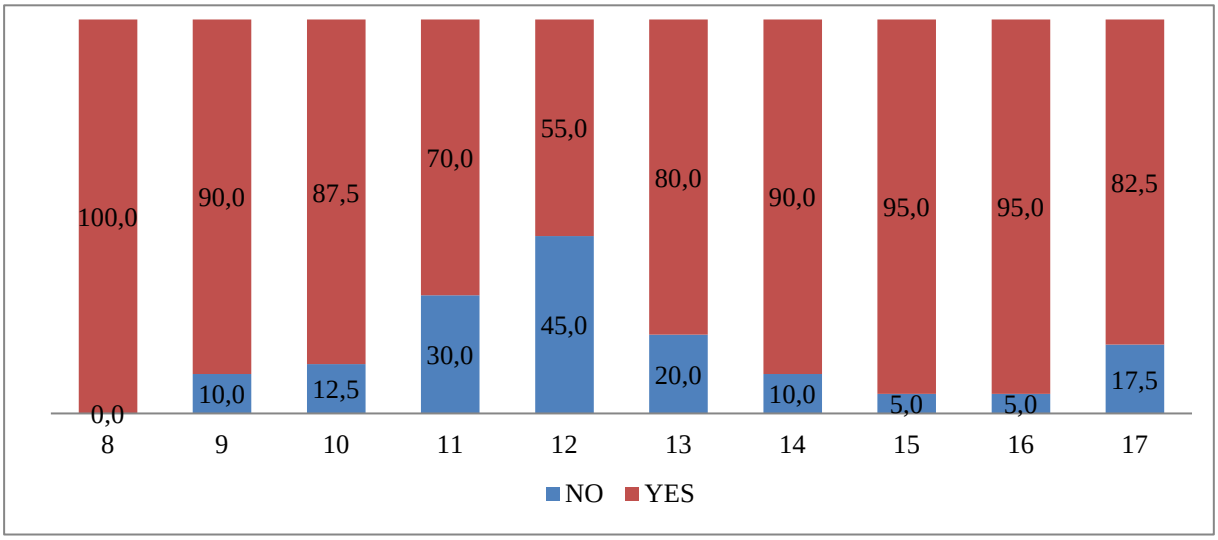

Figure 4. Consumption of sweetened foods.

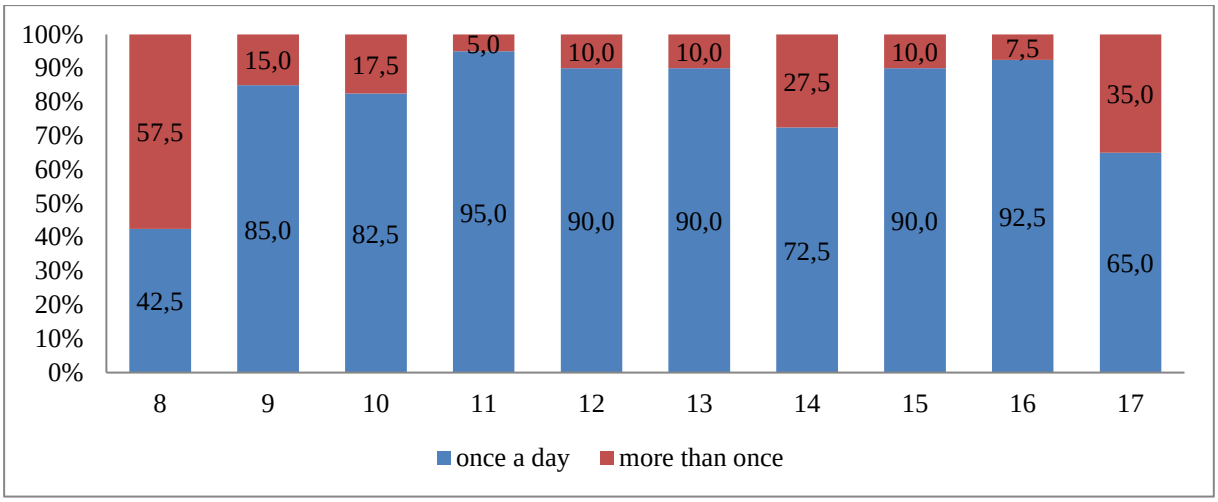

Figure 5. Frequency of intake of sweetened food and beverages.

The duration of contact and the frequency of intake of cariogenic carbohydrates also play an important role. This determines how many times the risk factor will affect the tooth structures. Therefore, the more frequent the consumption of this type of food and drink, the more often a drop in the pH value is observed and, accordingly, the greater the possibility of carious lesions. It has been established that at an early age, even when the first permanent molars erupt, a high percentage of children often consume more carbohydrates with high cariogenic potential. Consumption of juices is shown in **Figure 6.**

The frequency of carbohydrate food and drink intake is not in favor of children with a single intake. When comparing the high percentage of multiple intake (in eight-year-olds) with the results of dental status studies, it can be concluded that already in the first age group a low percentage of carious lesions of milk teeth is observed. In nine-year-olds, the percentage of teeth with caries increases, and the increase continues to be present in ten-year-olds. The worrying fact is that at the same time, carious lesions are observed in permanent teeth, with the DMFT index in eight-year-olds having a low percentage, which increases dramatically in nine-year-olds.

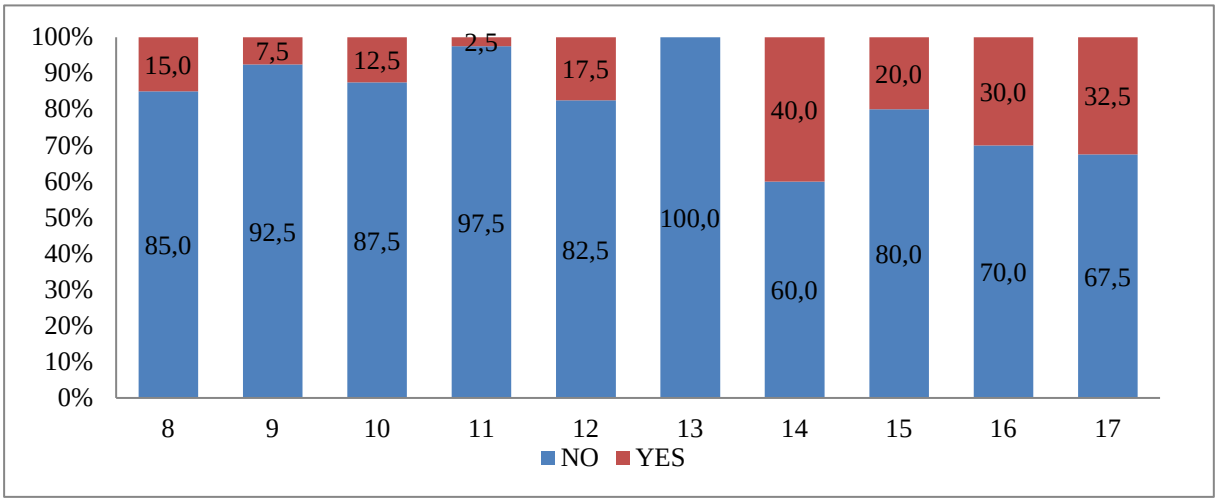

Figure 6. Consumption of juices

Discussion

This study found a direct correlation between sports as a professional activity in the study of child soccer players and low oral hygiene index of Green Vermillion (OHI-S). Several studies have found similar results to ours. Several authors have reported that DMFT, the index it tracks, has significantly higher values in children who actively participate in sports compared to children who do not actively participate in sports [4, 5, 6].

Sport as a risk factor contributing to the development of caries has also been described in the studies of Bryant et al. and Frese et al. They believe that active or professional sports involve a clear predisposition to dental caries and erosion [4, 7].

May Tenabe et al. believe that the identified carious and erosive lesions are the result of drying of the oral mucosa caused by dehydration, increased intake of sports drinks and foods [8]. Reduced salivary secretion and increased salivary viscosity lead to reduced oral carbohydrate clearance and thus increase the risk of developing carious lesions [9, 10, 11, 12, 13]. This thesis is confirmed by other studies that have found a significant positive relationship between salivary flow and salivary pH [14].

These results coincide with and confirm the data obtained by our team (high-carbohydrate diet and intake of energy and hydration drinks with high carbohydrate content, reduced oral hygiene, reduced salivation).

Broad EM et al. [15] found that recommended foods and drinks with a high percentage of carbohydrate content and high energy potential for child athletes lead to an increased risk of developing carious lesions. Individuals with increased carbohydrate intake showed a greater number of carious teeth [16].

De Sant'Anna GR [17] in his study conducted on 18 girls aged 13 to 19 years, he found a similarly high caries activity, with a mean DMFT value of 8, which can be considered significantly high. Even more impressive results were found by de Cárdenas [18], who studied 209 professional athletes in Cuba, with 75.12% of the participants having carious lesions.

Conclusion

Knowing, monitoring, and reducing risk factors is crucial for oral health. The consequences of not understanding and ignoring these risk factors can adversely affect the oral and general health of children, as well as their sports performance. The key to preventing these consequences of sports on oral health is the preparation and adherence to optimized diets, hydration, and improved oral hygiene in these children.

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