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I. Surgical Session

Abdominal actinomycosis complicated with intestinal obstruction, peritoneal abscess, and extensive abdominal fibrosis – a rare case presentation

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Background: Abdominal actinomycosis is an uncommon chronic suppurative disease caused by *Actinomyces israelii*, which results in infiltrative abdominal mass lesions, which are often indistinguishable from malignancy. We **aim** to present a rare case of actinomycosis complicated with intestinal obstruction, terminal ileum perforation and extensive abdominal fibrosis. **Case presentation:** A 71-year-old man presented with squeezing abdominal pain. Palpation of the abdomen revealed acute peritoneal signs. A computed tomography scan of the abdomen revealed distended intestinal loops, an abscess in the right lower quadrant, abdominal and pelvis mass lesions. Laparotomy was performed and as an operative finding it was diagnosed as inflammatory process of the terminal ileum with perforation of the intestine, right iliac fossa abscess, and mass lesion that infiltrated the anterior abdominal wall, retroperitoneal space and pelvis which was indistinguishable from malignancy. Resection of the inflamed intestinal segment and drainage of the abdominal abscess was performed and a specimen from the mass lesion was also obtained. Histological examination of the resected intestinal segment and abdominal mass revealed a colony of actinomyces and a mixed inflammatory response. **Conclusion:** We present a rare case of abdominal actinomycosis that is complicated with intestinal obstruction, peritoneal abscess, and extensive abdominal fibrosis. The clinical interest of the presented case is a severe progression of this uncommon chronic suppurative infectious disease.

Keywords: abdominal actinomycosis, intestinal obstruction, mass lesion, peritoneal abscess

Introduction

Actinomycosis arises from gram-positive, non-acid-fast, microaerophilic, and anaerobic filamentous bacteria in the *Actinomyces* species, primarily *Actinomyces israelii* (1). Other identified species that can cause human diseases include *A. bovis*, *A. ericksonii*, *A. naeslundii*, *A. viscosus*, and *A. odontolyticus* (2).

This bacterium is typically saprophytic and colonises mainly the upper respiratory tract, gastrointestinal tract, and female genital tract. It commonly infects organs within the pelvis, intra-abdominal area, and lungs (3). The organism remains asymptomatic unless barriers are disrupted, allowing bacterial dissemination through surrounding tissues (4).

Case presentation

A 71-year-old man presented with squeezing diffuse abdominal pain of one day on set. Palpation of the abdomen established acute peritoneal signs. There was no history of abdominal surgery or abdominal trauma.

Blood chemistry tests revealed the mean numbers of WBC $24.97 \times 10^9/L$ and C-reactive protein (CRP) 169.0 mg/L. Thoraco-abdominal X-Rays presented distended intestinal loops with multiple air-fluid levels (fig. 1). A Computed tomography (CT) scan of the abdomen revealed free peritoneal air, distended intestinal loops, exudate in the right iliac fossa, thickening of the wall of the terminal ileum, thickening of the abdominal wall of the right lower quadrant (fig. 2).



Figure 1. Thoraco-abdominal X-Ray: Abdominal CT scans dilated intestinal loops with multiple air-fluid levels).

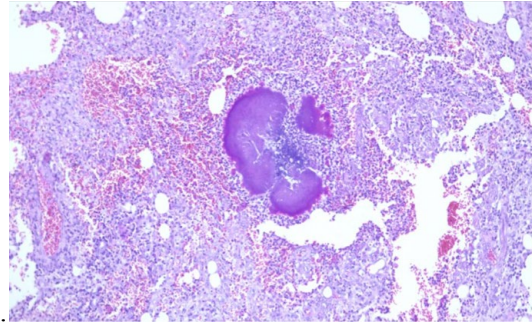


Figure 2. Abdominal CT scans show dilated small bowel loops, thickening of the small-bowel wall and anterior abdominal (pointed with red arrows).

Emergency Laparotomy was performed and as an operative finding, inflammatory process of the terminal ileum with perforation of the intestine, right iliac fossa abscess and mass lesion that infiltrates anterior abdominal wall, retroperitoneal space and pelvis was established. Grossly, the mass lesion was indistinguishable from malignancy. Resection of the affected part of the terminal ileum was performed by creating ileostomy and drainage of the abdominal abscess. The incisional biopsy specimen was obtained from the abdominal wall mass lesion as well.

Histological examination of the resected intestinal segment and abdominal mass revealed colony of actinomyces and mixed inflammatory response (fig. 3A; 3B).

3A.



3B.

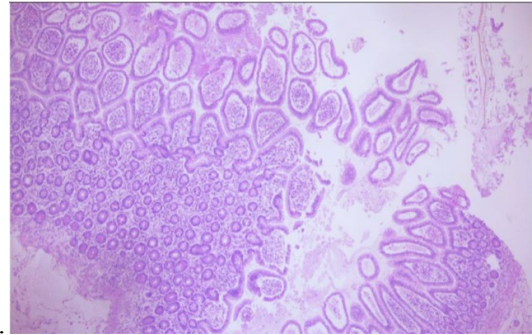


Figure 3A&3B. A histopathological sample of anterior abdominal wall: focus of actinomycosis with surrounding chronic inflammatory cells and foamy macrophages (haematoxylin–eosin stain, magnification: 2A – $\times 100$; 2B – $\times 200$).

Discussion

Actinomycosis was initially documented in 1846 by Bradshaw, who described a patient with the formation of an abnormal passage between a mass in the right iliac fossa and the skin (5). Actinomycosis commonly impacts the oral cavity, female genital tract, and bronchial system. This categorises actinomycosis into cervicofacial, thoracic, and abdominopelvic types based on the location of involvement (6). Any form of damage to mucosal barriers throughout the body caused by factors such as endoscopic procedures, surgeries, aspiration events, or chronic inflammatory diseases can serve as predisposing factors for penetration by *Actinomyces* bacteria. Various forms of immunosuppression, like leukemia, lymphoma, renal insufficiency transplants, and diabetes, have been shown to facilitate this process (7).

The breakdown of the mucosal barrier can result in infection. The initial acute

phase is frequently overlooked during diagnosis, leading to the chronic phase. In this stage, the organism grows slowly and continuously, spreading through different tissue planes and forming a sinus tract with surrounding structures. This can lead to spontaneous healing followed by reoccurrence, resulting in the formation of a densely fibrotic lesion often described as “wooden” (8, 9). *A. israelii* (the primary causative agent) becomes saprophytic under anaerobic conditions and penetrates the damaged mucosa. Once they invade tissues, they develop a persistent granulomatous infection characterised by the formation of small clumps known as sulfur granules due to their yellow colour (10, 11).

Actinomyces inhabits typically the colon, particularly areas with stagnant conditions such as the cecum and appendix, leading to extensive abdominal fibrosis and chronic inflammatory processes in the intestine. Its most commonly seen in the terminal ileum and right side of the colon, which increases the risk for intestinal obstruction and perforation (10, 11). The clinical symptoms presented by actinomycosis are characterised by the contiguous spread of infection as well as suppurative and granulomatous inflammation. The invasion of host tissue triggers an inflammatory response that can lead to the development of multiloculated abscesses and pseudo tumours. It leads to the formation of multiple abscesses and sinus tracts, resulting in dense fibrosis and a hard mass. The pathogenesis of abdominal actinomycosis is not fully explained, but it is believed to occur through blood-borne infection or ingestion of substance like fish bone, affecting intrabdominal organs. Clinically, abscesses associated with actinomycosis can be misdiagnosed as malignant tumours, often resulting in a delayed or missed diagnosis until surgery takes place (12).

Although CT is an effective diagnostic method for visualising actinomycotic lesions, it does not definitively confirm the diagnosis (13). Radiological findings are non-specific and closely resemble those seen in Crohn's disease, intestinal tuberculosis, or occasionally a malignant tumour with cavitation (14). The ultimate diagnosis relies on isolating actinomycetes from pus or examining a suspicious mass histologically. In cases of suspected actinomycosis, CT-guided aspiration with a core needle biopsy serves as a valuable diagnostic approach. However, pus cultures rarely identify actinomycetes; thus, these lesions are often misdiagnosed before surgery as malignant tumours (15).

Role of Histopathology and microbiological analyses is crucial in making the diagnosis. A definitive determination is made through the identification of sulphur granules, the culture of *Actinomyces* from a surgical specimen, or the needle aspiration of an abscess. Endoscopic biopsies typically offer limited insights and only show chronic inflammation without sulphur granules, mainly due to the shallow tissue sampling. Sulphur granules indicate colonies of *Actinomyces*, but they are not exclusive to actinomycosis and can also be present in nocardiosis, botryomycosis, and *Aspergillus* infections (16). The clinical presentation of actinomycosis can resemble that of other chronic conditions, such as granulomatous diseases like Crohn's disease or malignant growths as well (19, 20). Therefore, CT-guided fine needle aspiration may aid in diagnosing this condition; however, it cannot be confirmed specifically and it can be challenging to obtain adequate samples, particularly when the small or large bowel is affected. Additionally, these samples may not fully represent the active disease area, leading to false-negative histology (17). Notably, this condition is usually diagnosed

postoperatively during pathological examination of the surgical specimen, unless there are pre-existing risk factors directly correlating to condition. In a nutshell an accurate diagnosis can only be made postoperatively based on the histopathological examination of a surgically removed specimen (15).

A more precise preoperative diagnosis would allow for the use of antibiotic therapy, with surgical intervention specifically reserved only for complications. However, accurately diagnosing actinomycosis before surgery is difficult due to the non-specific findings from imaging studies and may require percutaneous ultrasound-guided fine-needle aspiration in cases of an abdominal abscess. For example, CT scan results showing an infiltrating mass in the abdomino-pelvic area without well-defined borders and increased uneven contrast may suggest actinomycosis when combined with symptoms such as fever, elevated white blood cell count, or underlying risk factors. But surgery is required for final treatment of complications and confirmation of diagnosis due to its size and characteristics, along with the risk of malignancy (21).

Looking back at our patient's case, a CT-guided biopsy might have facilitated obtaining a more precise histological diagnosis. In spite of this option, as there were concerns about potential malignancy, urgency of the condition and uncertainties regarding guided biopsy yield, surgery was the chosen method.

This particular case is uncommon due to several factors. Primarily, our patient did not exhibit any risk factors, which resulted in the exclusion of actinomycosis from the differential diagnosis. Furthermore, he presented with widespread abdominal pain that manifested suddenly for a day. Upon palpation of the abdomen, acute peritoneal signs were noted, resembling symptoms of an acute abdominal condition.

In our case, specific contributors to colon perforation remain uncertain; however, it can be hypothesised that increased pressure from a right iliac fossa abscess and subsequent penetration and mass lesion pressure on the anterior abdominal wall, retroperitoneal space, and pelvis, along with colorectal stricture, likely led to colon perforation accompanied by acute abdomen symptoms.

Conclusion

Abdominal actinomycosis is a rare condition that can resemble various intra-abdominal issues, including acute inflammatory conditions. It should be considered in the differential diagnosis of abdominal diseases with inflammatory characteristics, especially when imaging shows solid tumour like features with non-uniform focal areas and invasion of nearby tissues. Procedures such as CT-guided biopsy/FNA and biopsy may help in establishing a potential diagnosis but cannot be concluded. The final diagnosis usually requires post-operative assessment due to the potential risk of malignancy, as confirmation typically relies on histological analysis of samples obtained during surgical exploration.

Bibliography

1. Tarzi M, Douedari A, Aldakhil R, Danial AK, Al-Haj A. Case report: Actinomycosis of the abdominal wall. *Journal of Surgical Case Reports*. 2021 May 1;2021(5). doi:10.1093/jscr/rjab171
2. A; YMHI. Actinomycosis: A differential diagnosis for appendicitis. A case report and review of the literature [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/17560191/>
3. J; HDJC. Treatment strategy for pelvic actinomycosis: Case report and review of the literature [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/10725583/>
4. Pitot D;De Moor V;Demetter P;Place S;Gelin M;El Nakadi I; Actinomycotic abscess of the anterior abdominal wall: A case report and literature review [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/18807608/>

5. Cope VZ. Visceral actinomycosis. *BMJ*. 1949 Dec 10;2(4640):1311–6. doi:10.1136/bmj.2.4640.1311
6. Sung HY, Lee IS, Kim SI, Jung SE, Kim SW, Kim SY, et al. Clinical features of abdominal actinomycosis: A 15-year experience of a single institute [Internet]. U.S. National Library of Medicine; 2011 [cited 2024 Apr 15]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC3124725/
7. Dominguez DC, Antony SJ. Actinomyces and Nocardia infections in immunocompromised and no immunocompromised patients [Internet]. U.S. National Library of Medicine; 1999 [cited 2024 Apr 15]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC2568306/
8. Thomas A R, kasper, Dan(eds), Longo, Stephen, Hauser, et al. Actinomycosis and Whipples disease [Internet]. [cited 2024 Apr 15]. Available from: https://accessmedicine.mhmedical.com/content.aspx?legacysectionid=kas_ch200
9. Valour F, Sénéchal A, Dupieux C, Karsenty J, Lustig S, Breton P, et al. Actinomycosis: Etiology, clinical features, diagnosis, treatment, and management [Internet]. U.S. National Library of Medicine; 2014 [cited 2024 Apr 15]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC4094581/
10. JE; H. Pleuropulmonary manifestations of actinomycosis and nocardiosis [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/3062727/>
11. M H, J S. Characterization of actinomycotic granules by architecture and staining methods [Internet]. [cited 2024 Apr 15]. Available from: <https://www.wikidata.org/wiki/Q43782294>
12. R; A-CR-R. Perianal actinomycosis--a complication of a fistula-in-ano. report of a case [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/8168418/>
13. Ha HK;Lee HJ;Kim H;Ro HJ;Park YH;Cha SJ;Shinn KS; Abdominal actinomycosis: CT findings in 10 patients [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/8372760/>
14. Triantopoulou C, Van der Molen A, Van Es AC, Giannila M. Abdominopelvic actinomycosis: Spectrum of imaging findings and common mimickers [Internet]. U.S. National Library of Medicine; 2014 [cited 2024 Apr 15]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC4001438/
15. Pusiol T, Morichetti D, Pedrazzani C, Ricci F. Abdominal-pelvic actinomycosis mimicking malignant neoplasm [Internet]. Hindawi; 2011 [cited 2024 Apr 15]. Available from: <https://www.hindawi.com/journals/idog/2011/747059/>
16. Wong VK, Turmezei TD, Weston VC. Actinomycosis [Internet]. British Medical Journal Publishing Group; 2011 [cited 2024 Apr 15]. Available from: <https://www.bmj.com/content/343/bmj.d6099>
17. B; GSP. Case report: Percutaneous drainage of periappendiceal actinomycosis [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/1773565/>
18. Pamathy G, Jayarajah U, Gamlaksha DS, Constantine R, Banagala ASK. Abdominal actinomycosis mimicking a transverse colon malignancy: A case report and review of the literature - journal of medical case reports [Internet]. BioMed Central; 2021 [cited 2024 Apr 15]. Available from: <https://jmedicalcasereports.biomedcentral.com/articles/10.1186/s13256-021-02812-7>
19. Willms A, Schaaf S, Güsken C, Waldeck S, Schwab R. Abdominelle aktinomykose: Eine Seltene differenzialdiagnose zu kolonkarzinom und morbus crohn [Internet]. © Georg Thieme Verlag KG; 2014 [cited 2024 Apr 15]. Available from: <https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0034-1366420>
20. Pitot D;De Moor V;Demetter P;Place S;Gelin M;El Nakadi I; Actinomycotic abscess of the anterior abdominal wall: A case report and literature review [Internet]. U.S. National Library of Medicine; [cited 2024 Apr 15]. Available from: <https://pubmed.ncbi.nlm.nih.gov/18807608/>
21. Könönen E, Wade WG. Actinomyces and related organisms in human infections [Internet]. U.S. National Library of Medicine; 2015 [cited 2024 Apr 15]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC4402957/

Arthroscopic treatment for cartilage damage of the knee

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Purpose: To present arthroscopic treatment as a method of minimally invasive approach for initial cartilaginous changes of the knee joint. **Material and methods:** For the period from June 2013 – July 2023, in the Clinic of Orthopedics and Traumatology at the University Hospital “St. George” Plovdiv were performed diagnostic and therapeutic arthroscopy of the knee joint in 563 patients. 382 women and 181 men, aged from 43 to 72 years old. Joe Tippet’s classification was used to grade the cartilage changes found in the knee joint during the minimally invasive procedure. In end-stage cartilage damage, it was mainly applied the abrasive arthroplasty technique. **Results:** 360 patients diagnosed with grade I – VI cartilage damage had complete recovery of the knee joint function. In another 152 of them with established grade VII and VIII, the condition improved with resolution of joint pain, increased range of motion, and resolution of chronic synovitis. In 42 of the patients there was no change in the joint condition, while in 9 others the condition even worsened. **Conclusion:** Arthroscopy of the knee joint is a minimally invasive surgical intervention aimed at preventing and preserving the joint from progression of osteoarthritis of the knee, as well as delaying the onset of irreversible bone changes in the joint.

Keywords: arthroscopic procedure, abrasive arthroplasty, cartilage damage

Introduction

Degenerative joint lesions represent a serious problem in which the diagnosis, histological picture and radiological changes are well studied. Once started, they progress gradually. When the options of conservative treatments have diminished or been exhausted, patients begin to seek surgical intervention (1). This late referral of patients becomes a reason for missing the most convenient time for surgical intervention. Nowadays, with the introduction and development of new methods and technologies, the surgical treatment of the joints affected by the arthrosis process takes the leading place after the healing methods. Its effectiveness has also been demonstrated in the treatment of osteoarthritis of the knee joint (2). The disease at a young age (under 35 years) is usually rare. The main reasons for this can be highlighted as the following factors: increasing average age of the population; increased activity at any age, and also sports activity; overweight.

Objective

To present arthroscopic treatment as a

method of minimally invasive approach for initial cartilage changes of the knee joint.

Material and methods

For the period from June 2013 to July 2023 at the Clinic of Orthopaedics and Traumatology at the University Hospital “St. George” Plovdiv we have performed diagnostic and therapeutic arthroscopy of the knee joint in 563 patients. There were 382 women and 181 men, aged between 43 and 72 years (average 63 years). We used Joe Tippet’s classification to grade the cartilage changes found in the knee joint during the intervention (3). Intraoperatively, when diagnosing end-stage cartilage damage, we applied the abrasive chondroplasty technique. Through the procedure we have solved the problem of the chondral damage (4). The mass introduction of arthroscopy and its continuous development has led to the development of new advanced concepts for the treatment of osteoarthritis based on minimally invasive surgery and “live” visualization of the joint. The Joe W. Tippet classification of chondral changes contains the following grades:

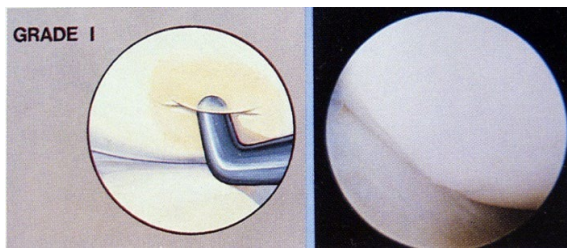


Figure 1. Grade I Softening of the articular cartilage demonstrated by palpation with an arthroscopic probe

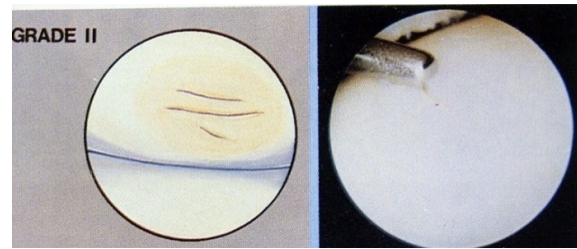


Figure 2. Grade II Linear fissures, which can be penetrated up to a few millimetres with a probe

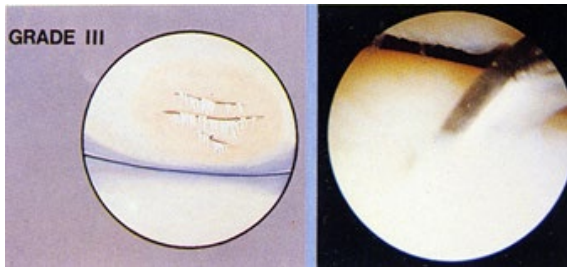


Figure 3. Grade III Linear fissures from which vertically descending fibers of eroded cartilage, called “tongues”

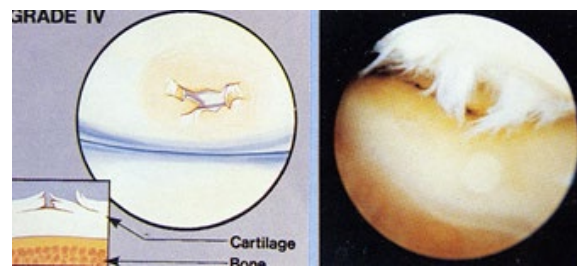


Figure 4. Grade IV Presence of a central cartilage defect with loss of substance from the thickness (20–50%) and “tongues” hanging from the edges of the defect.

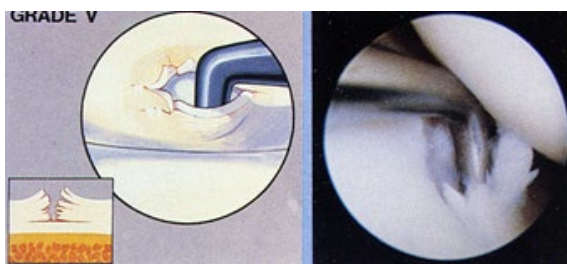


Figure 5. Grade V Cartilage defect with 50 to 90% thickness loss and hanging “tongues” at the edge of the defect.

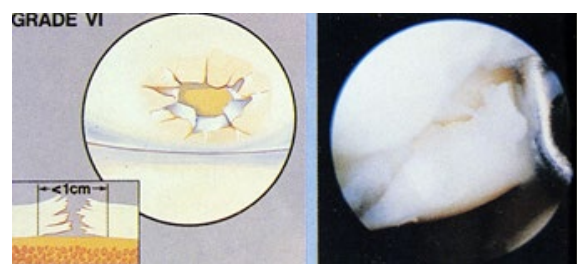


Figure 6. Grade VI Defect with 100% cartilage loss and exposed subchondral bone, but the defect is no more than 1 cm

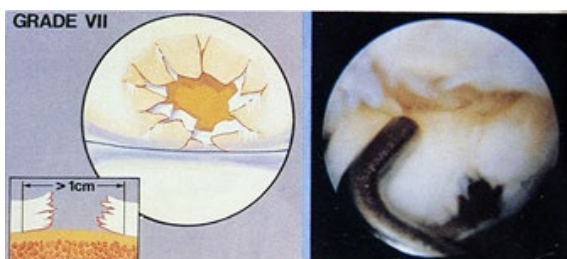


Figure 7. Grade VII A defect measuring more than 1 cm

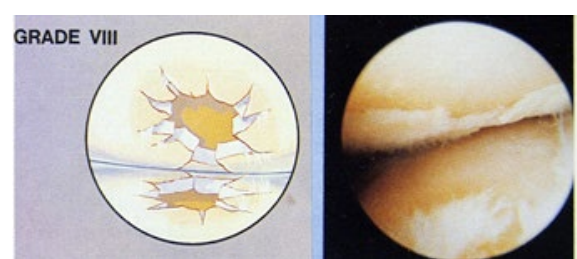


Figure 8. Grade VIII Defects of varying size and presence of exposed sclerotic erupted bone

In patients diagnosed with grade I, II, III cartilage changes we applied cleaning to healthy structure of the damaged cartilage by “Shaver” with 4.5 mm knife – full radius – Synovial resector, followed by lavage with 1000 ml Ringer.

In case of IV, V, VI degree of

chondropathy, after treatment of the cartilage to a healthy as described above, we perform the so-called microfractures in the bone to a depth of 2–3 mm, using chondropics, until bleeding from the avulsion. The purpose of this procedure is the formation of fibrous cartilage (Fig. 9).

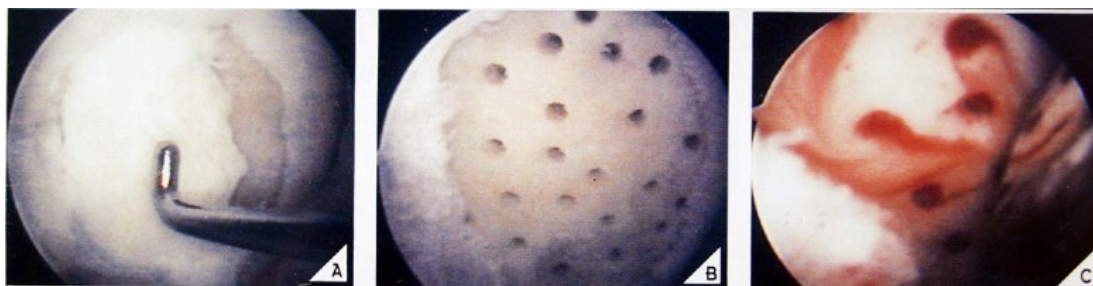


Figure 9. Perforations in the bone until bleeding

In patients with advanced arthrosis – grade VII and VIII of the knee with visible radiographic “collapse” most often of the medial compartment (medial femoral condyle, medial tibial condyle) we applied the so-called abrasive chondroplasty using the Lany Jonson method (4). We have performed “revitalization” on the cartilage-exposed sclerotic and eburnated areas of the articular surfaces of the medial femoral and/or tibial condyles. Through the anteromedial arthroscopic approach via the “Shaver” with 4 mm oval bore are made multiple removals of the sclerotic bone at a depth of 3–4 mm at a distance of 2–3 mm from each other. This procedure is performed until bleeding (visible capillary bleeding) from the made “craters”. After that we have with 2000 ml Ringer and aspiration of bone “shred”. When degenerative meniscal lesions are diagnosed intraoperatively, we have performed a partial meniscectomy or meniscal suture, and when menisci are present, they are extirpated arthroscopically. In the early postoperative period we apply cryotherapy and analgesic therapy. From the second day after the intervention, active kinesitherapy (isometric exercises) is started on the extensor apparatus, which indirectly improves the revascularization of the revitalized area. The hospital stay in the clinic is three days. For patients with grade I, II, and III cartilage damage, we recommended weight bearing of the limb for a period of two weeks until suture removal. In those with grade IV, V and VI –

limb weight bearing for a period of one month, while in patients with grade VII and VIII degree of disability the weight bearing of the operated limb is for a period of 45 to 60 days. We have strictly adhered to the principles of Lany Jonson.

Results

Patients were followed for a period of one year. Both clinical and functional assessment of the arthroscopic knee joint was performed. We used scores from the modified Jönsson Insall scoring system (Knee Score/Functional Score) to assess preoperative and postoperative outcomes (5).

In 360 of the patients diagnosed with grade I–VI cartilage damage, complete recovery of knee joint function was achieved. In another 152 of them with established grade VII and VIII severity, the condition improved with resolution of joint pain, increased range of motion, and resolution of chronic synovitis. In 42 of the operated patients there was no change in the joint condition, while in 9 others the condition even worsened. We did not observe complications of general and local nature.

Discussion

In this study, we were guided by the principle of early diagnosis followed by early arthroscopic therapy. In this way, irreversible cartilage and bone changes are avoided, with the end result of disability or severe surgical interventions – periarticular osteotomies or

joint arthroplasty (6, 7). Degenerative osteoarthritis of the knee joint in its early stages is caught only arthroscopically by demonstrating collapse of articular cartilage and is demonstrated on radiography (facet projection) with a narrowed joint space from the medial compartment.

Conclusions

Arthroscopy of the knee joint is a minimally invasive surgical intervention that aims to prevent and preserve the joint from the progression of osteoarthritis of the knee, as well as delay the onset of irreversible bone changes in the joint.

References

1. Mahir L, Belhaj K, Zahi S, et al. Impact of knee osteoarthritis on the quality of life. *Ann Phys Rehabil Med* 2016;59s:e159.
2. Alkan BM, Fidan F, Tosun A, et al. Quality of life and self-reported disability in patients with knee

- osteoarthritis. *Mod Rheumatol* 2014;24:166–71.
3. Tippet W. J Classification of cartilaginous lesions Operative arthroscopy by I. B. Mc Ginty et al. 1991, 327-329
4. Dandy D.J Abrasion chondroplasty. *Arthroscopy* 1886: 2: 51-53
5. Jacobs C, Christensen Ch. Correlations between Knee Society Function Scores and Functional Force Measures *Clin Orthop Relat Res* 2009: 467:2414–2419
6. Khan M, Evaniew N, Bedi A, et al. Arthroscopic surgery for degenerative tears of the meniscus: a systematic review and meta-analysis. *CMAJ* 2014;186:1057–64.
7. Thorlund JB, Juhl CB, Roos EM, et al. Arthroscopic surgery for degenerative knee: systematic review and meta-analysis of benefits and harms. *BMJ* 2015;350:h2747.

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Morphologic changes in carpal tunnel syndrome

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Introduction: Carpal tunnel syndrome (CTS) is the most common compression neuropathy of the upper extremities. The median nerve is compressed under the flexor retinaculum due to primary and secondary changes. Primary CTS is polyetiological while the etiology of idiopathic CTS is unknown. Histologic changes in the subsynovial connective tissue of the flexor tenosynovium (SSCT) of patients with CTS have been investigated, but their clinical and prognostic importance is unclear. Very important is the fact that the retrospective design of many previous studies does not allow correct evaluation of the preoperative changes in relation to following therapy and prognosis. **Aim of the study:** The aim of the study were the histologic changes in the tenosynovial tissue in ten surgically treated patients for carpal tunnel syndrome and median nerve compression. Tissue samples of the synovial connective tissue under the median nerve were studied for pathologic changes. **Results and discussion:** The results showed reactive chronic synovitis, synovial hypertrophy, island-like fibrosis and hyalinosis. Histologically the samples were stained with Hematoxylin and Eosin, PAS reaction was performed for histochemical investigation with Shift method, and Van Gieson and Congo Red were used in order to rule out amyloidosis. **Conclusion:** We would suggest evaluation of the tenosynovial tissue under the flexor retinaculum after retinacular release. In case of inflammation, hypertrophy or mas effect, the surgeon must perform partial tenosynovectomy in order to decrease the volume of the carpal tunnel or to prevent the recurrence possibility.

Keywords: Carpal tunnel, histopathology, median nerve, synovium

Introduction

Carpal tunnel syndrome (CTS) is the most common neuropathy of the upper limbs, which affects approximately 3.8% of the general population and is more common in women than men¹. Several medical and non-medical factors influence the compression of the median nerve under the transverse carpal ligament (TSL). Occupational factors have been observed by Agnessa Kozak et al.² to play a considerable role in CTS etiology. Comorbidities, such as diabetes mellitus, vitamin deficiencies, rheumatoid arthritis and thyroid malfunction have been observed to contribute to both development and worse prognosis in patients with CTS³. If no definite cause is identified, the condition is classified as idiopathic CTS. While non-surgical treatment options such as corticosteroid injections or physical therapy are available, the literature prioritizes surgery as it shows significantly better clinical improvement overall^{4,5}.

Aim of the study

The aim of our study was to identify the histological changes in the tenosynovial tissue of patients, diagnosed with idiopathic CTS. Such studies have been done in the past, but their retrospective design does not allow exact evaluation of the preoperative changes, therapy and prognosis⁶. Furthermore, we aimed to make further contributions to other studies, that associated CTS with transthyretin amyloidosis (ATTR) and heart failure (HF)^{7,8}.

Materials and methods

A total of 10 patients underwent open surgery for carpal tunnel decompression. In all patients an electrophysiological study was performed preoperatively and their symptoms and previous medical conditions were examined. There were no detectable reasons for CTS, so it was classified as idiopathic. Samples were collected from the synovial connective tissue dorsal to the median nerve and were sent for pathological evaluation.

Histologically, besides staining the specimens with Hematoxylin-Eosin, histochemical examinations were performed using PAS reaction with Schiff's reagent, Van Gieson for collagen fibers, and Congo red to exclude amyloidosis.

Results

Reactive chronic synovitis, synovial hypertrophy, locular fibrosis and hyalinosis were found. No amyloid deposits were detected.

Figures 1–4 illustrate the most common changes in the subsynovial connective tissue.

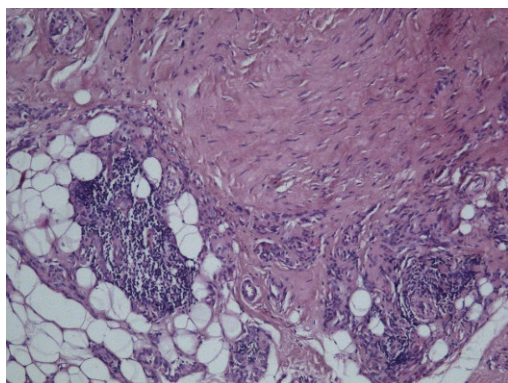


Figure 1. Chronic tenosynovitis, H&E, ×200

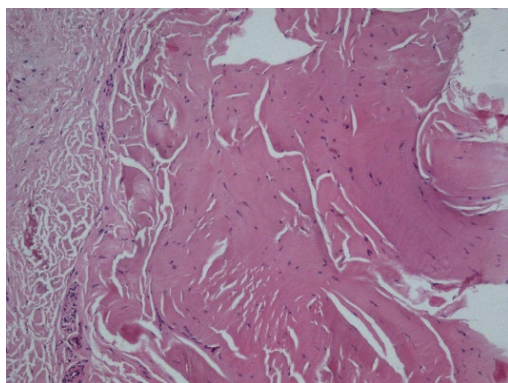
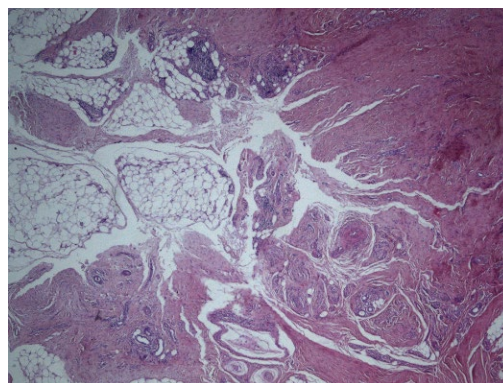


Figure 2. Fibro-hyaline nodules, H&E, ×100

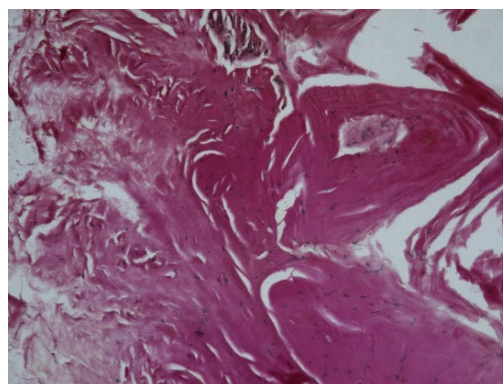
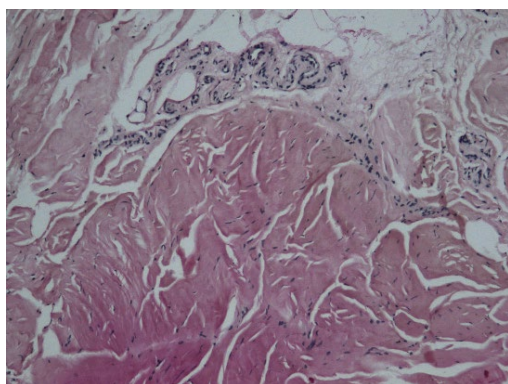
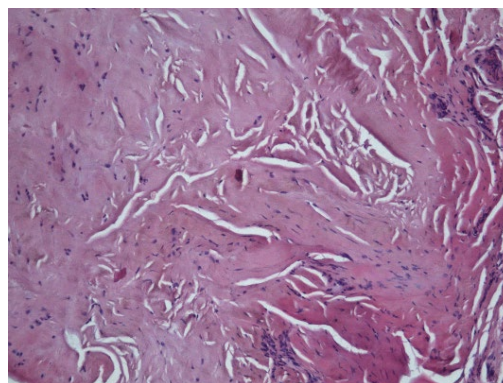


Figure 3. Van Gieson-positive for collagen fibers, fibro-hyaline nodules, ×100

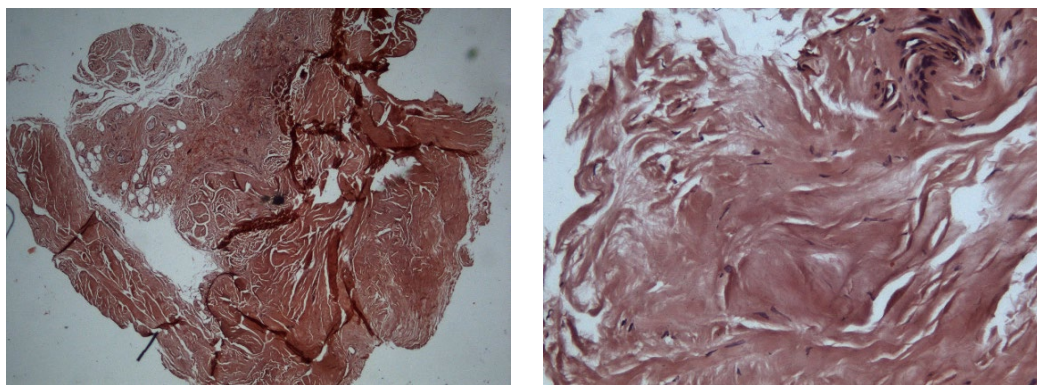


Figure 4. Congo Red-negative for amyloid depositions, fibro-hyaline nodules, $\times 100$

Discussion

The morphological alterations observed in carpal tunnel syndrome (CTS) typically stem from fibrosis of the synovial tissue beneath the nerve within the carpal tunnel, synovial hypertrophy, reactive synovitis, or the presence of fibro-hyaline nodules. Inflammatory responses are commonly minimal or absent, with little to no discernible fibroblast proliferation. Tenosynovectomy is warranted solely in cases of proliferative tenosynovitis (as seen in conditions such as rheumatoid arthritis, gout, and granulomatous infection), bacterial or fungal infections, or notably thickened tenosynovium due to amyloid deposition, particularly in patients undergoing dialysis.

Conclusion

The primary this study was to associate CTS with transthyretin amyloidosis (ATTR) and heart failure (HF). Although studies linking polyneuropathies, such as CTS, with ATTR have been conducted in the past^{7,8}, their amount is relatively low. Our objective is to further advance the research on this topic and contribute prognostic value to the existing literature. To date, amyloidosis has not been identified in our patients; nonetheless, our research work will proceed with the aim of providing valuable insight as we continue our investigation.

References

1. Harinesan N, Silsby M, Simon NG. Carpal tunnel syndrome. *Handb Clin Neurol.* 2024;201:61-88. doi: 10.1016/B978-0-323-90108-6.00005-3. PMID: 38697747.
2. Kozak A, Schedlbauer G, Wirth T, Euler U, Westermann C, Nienhaus A. Association between work-related biomechanical risk factors and the occurrence of carpal tunnel syndrome: an overview of systematic reviews and a meta-analysis of current research. *BMC Musculoskelet Disord.* 2015 Sep 1;16:231. doi: 10.1186/s12891-015-0685-0. PMID: 26323649; PMCID: PMC4553935.
3. Nordstrom DL, Vierkant RA, DeStefano F, Layde PM. Risk factors for carpal tunnel syndrome in a general population. *Occup Environ Med.* 1997 Oct;54(10):734-40. doi: 10.1136/oem.54.10.734. PMID: 9404321; PMCID: PMC1128928.
4. Verdugo RJ, Salinas RS, Castillo J, Cea JG. Surgical versus non-surgical treatment for carpal tunnel syndrome. *Cochrane Database Syst Rev.* 2002;(2): CD001552. doi: 10.1002/14651858.CD001552. Update in: *Cochrane Database Syst Rev.* 2003;(3): CD001552. PMID: 12076416.
5. Jarvik JG, Comstock BA, Kliot M, Turner JA, Chan L, Heagerty PJ, Hollingworth W, Kerrigan CL, Deyo RA. Surgery versus non-surgical therapy for carpal tunnel syndrome: a randomised parallel-group trial. *Lancet.* 2009 Sep 26;374(9695):1074-81. doi: 10.1016/S0140-6736(09)61517-8. PMID: 19782873.
6. Chang CY, Beduschi MG, Pacheco TA, de M Chang CC. Carpal Tunnel Syndrome: Retrospective Analysis of 60 Consecutive Cases of Ambulatory Surgical Treatment With Local Anesthesia. *Hand (N Y).* 2020 Jul;15(4):509-513. doi: 10.1177/1558944718813721. Epub 2018 Dec 2. PMID: 30501506; PMCID: PMC7370391.
7. Donnelly JP, Hanna M, Sperry BW, Seitz WH Jr. Carpal Tunnel Syndrome: A Potential Early, Red-Flag Sign of Amyloidosis. *J Hand Surg Am.* 2019 Oct; 44(10):868-876. doi: 10.1016/j.jhsa.2019.06.016. Epub 2019 Aug 7. PMID: 31400950.
8. Fosbøl EL, Rørth R, Leicht BP, Schou M, Maurer MS, Kristensen SL, Kober L, Gustafsson F. Association of Carpal Tunnel Syndrome With Amyloidosis, Heart Failure, and Adverse Cardiovascular Outcomes. *J Am Coll Cardiol.* 2019 Jul 9;74(1):15-23. doi: 10.1016/j.jacc.2019.04.054. PMID: 31272537.

Review of the anatomical variations of the median nerve in the carpal tunnel: a concise overview of literature

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Introduction: Carpal tunnel syndrome is the most common entrapment neuropathy. Median nerve compression due to increased pressure in the carpal tunnel leads to impaired function of the hand and discomfort and paresthesia. Surgical division of the transverse carpal ligament is a method of choice for the treatment of the condition due to common conservative treatment failure. **Aim of the study:** This paper aims at reviewing the most important anatomic variations of the median nerve in the carpal tunnel and their implication on surgical treatment of the disease. **Material and methods:** A review of literature was performed and the median nerve variations were described and summarized. Surgical importance and influence on treatment outcome were emphasized. **Results and discussion:** Different results have been reported on the frequency of median nerve variations. They are summarized in Lanz classification and the clinical importance of the different groups for treatment outcome is discussed. **Conclusion:** Anatomic variations of the median nerve in the carpal tunnel are frequent and may become a major obstacle on treatment outcome in patients who undergo surgery. Detailed knowledge on related anatomy is extremely important in every day surgical practice.

Keywords: anatomic variations, median nerve, carpal tunnel, surgery, complications

Introduction

Carpal tunnel syndrome is the most common upper limb entrapment neuropathy¹. Median nerve compression due to increased pressure in the carpal canal leads to impaired function of the hand and paresthesia². Surgical division of the transverse carpal ligament (TSA) is the treatment of choice for the vast majority of orthopaedic surgeons due to common conservative treatment failure^{3,4}. An adept doctor conducting carpal tunnel decompression must possess a comprehensive understanding of the diverse anatomical configurations of the median nerve within the wrist.

Aim of the study

This paper aims at reviewing the most important anatomic variations of the median nerve in the carpal tunnel and their implications on surgical treatment of the disease.

Materials and methods

A comprehensive review of the literature was performed and the median nerve variations at the wrist were summarized and evaluated. Surgical importance and influence on treatment outcome were emphasized.

Results

When comparing different meta-analyses, there are differences in the frequency and the type of variations of the median nerve. Overall, the Lanz classification is of the highest clinical and surgical importance⁵. Henry BM et al⁶ report rates of the extraligamentous, subligamentous, and transligamentous courses respectively being 72%, 13.5%, and 11.3%. The prevalence of Lanz group 2, 3, and 4 are 4.6%, 2.6%, and 2.3%. Lanz U on the other hand reports rates of 46%, 31%, and 23% respectively⁵.

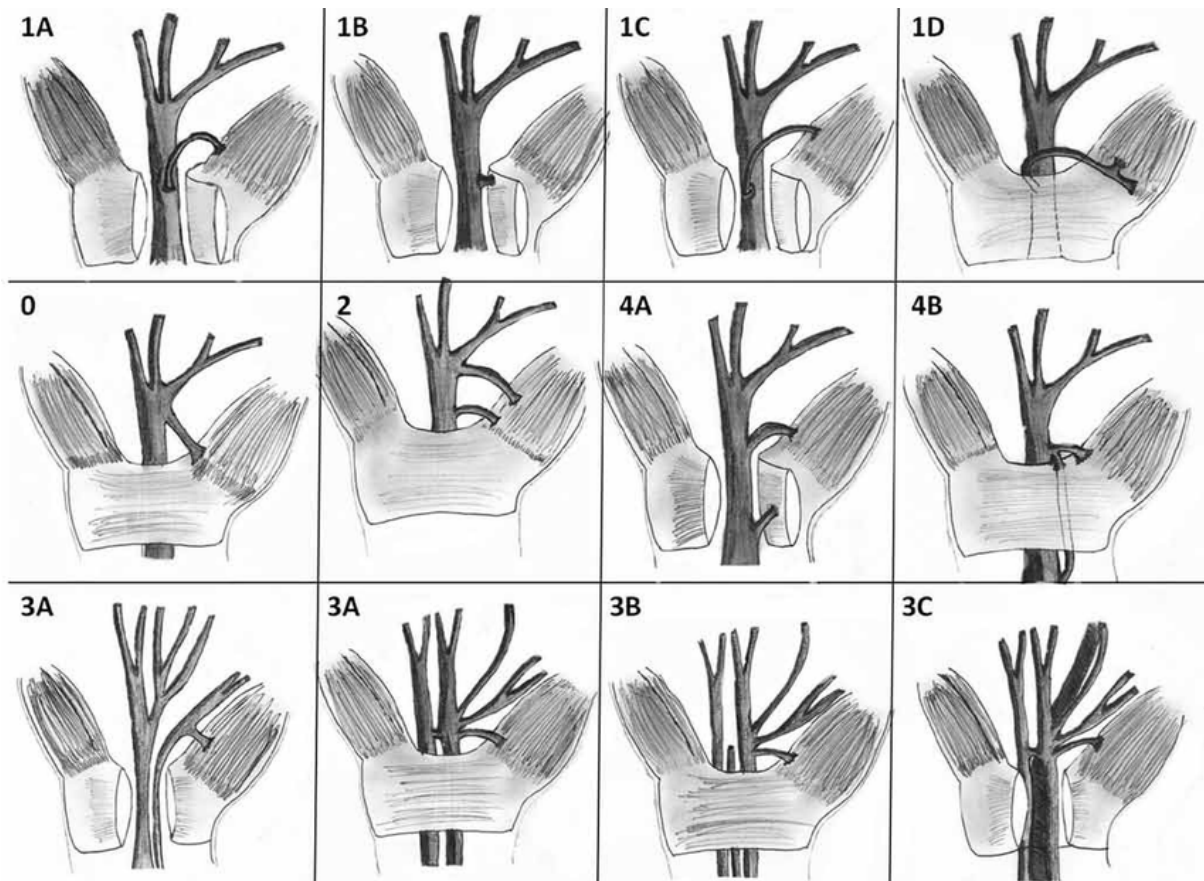


Figure 1. Lanz classification of the median nerve variations at the wrist^{7,8}. Group I, Thenar branch variations; 1A: subligamentous; 1B: transligamentous; 1C: ulnarwards; 1D: supraligamentous. Group 0, extraligamentous thenar branch. Group II, distal accessory thenar branch. Group IV, proximal accessory thenar branch; 4A: running directly in the thenar muscles; 4B: joining another branch. Group III, high division of the median nerve; 3A: without an artery or muscle; 3B: with artery; 3C: with lumbrical muscle.

Of equal importance and surgical implications are the anastomoses of the median and ulnar nerve at the wrist⁹. The Riche-Cannieu anastomosis (RCA) exists at the hand and is a connection between the recurrent branch of the median nerve and the deep branch of the ulnar nerve. The literature suggests that it is prevalent in 55% of the general population and that it is both a sensory and motor anastomosis^{10,11}.

The Berrettini Anastomosis (BA) is the most common median and ulnar nerve interconnection. Roy et al¹² estimate a prevalence of around 60%. BA is most commonly bilateral and is believed to be a purely sensory anastomosis. The ulnar nerve can provide sensory supply to digits 2 and 3 via a communicating branch.

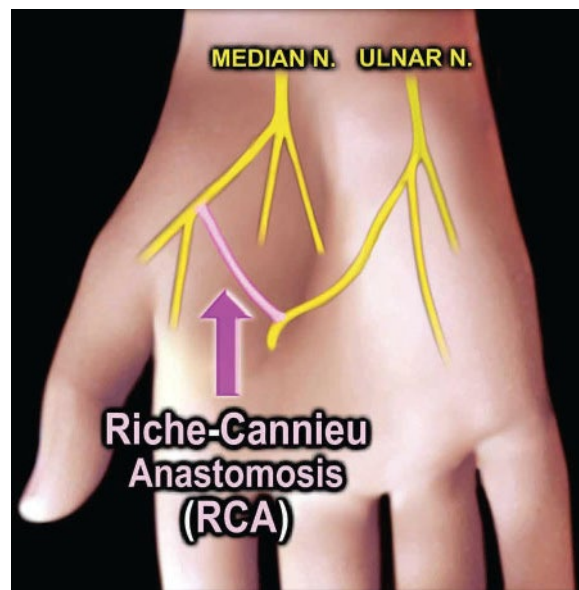


Figure 2. Riche-Cannieu anastomosis⁹

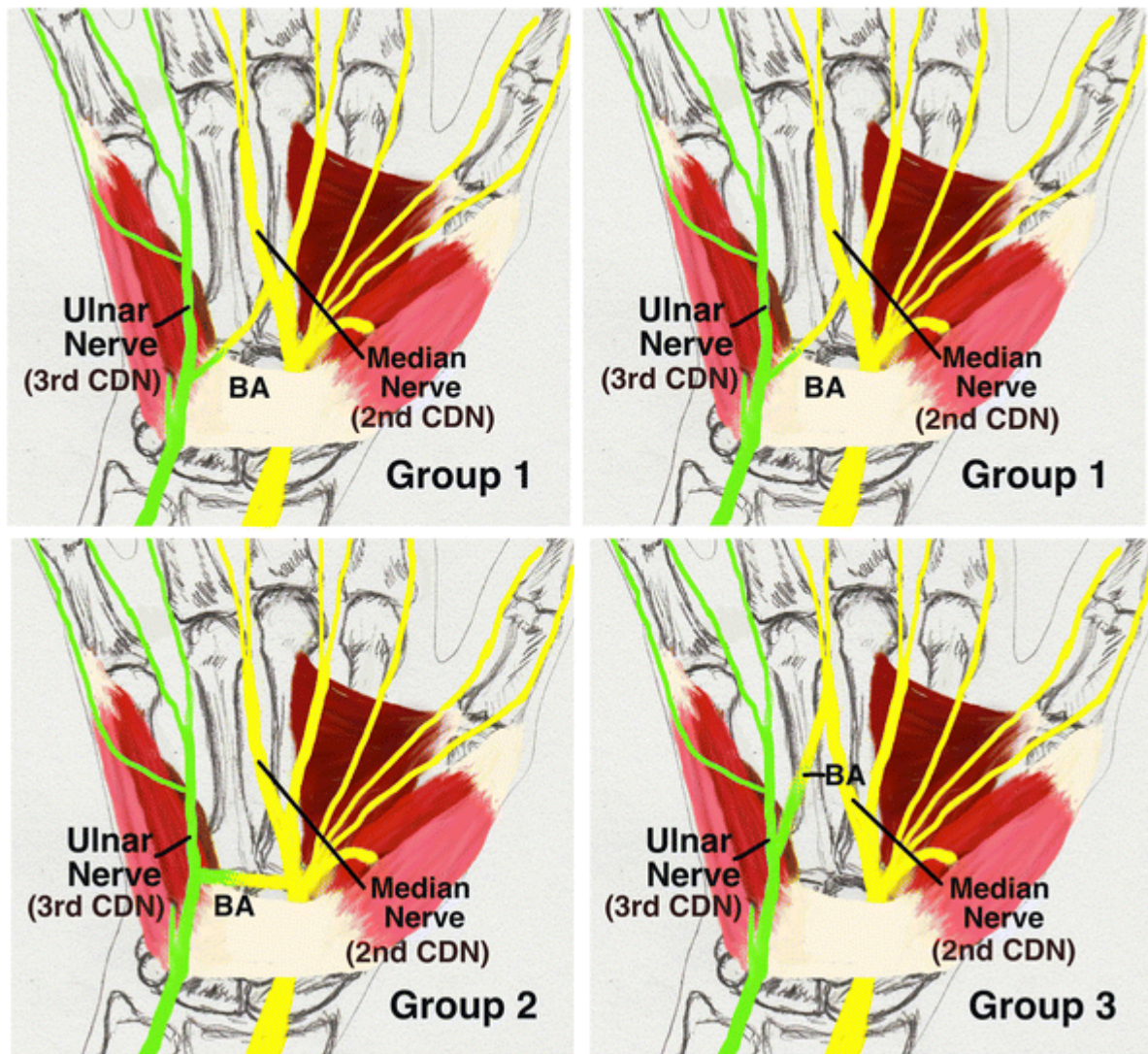


Figure 3. Berrettini anastomosis⁸

Discussion and conclusion

The vast majority of differences of the course and anastomoses of the median nerve at the wrist show that great caution and deep anatomical knowledge are required when treating wrist injuries and neuropathies. When surgically opening the carpal canal, the literature suggests and ulnar side approach⁵.

References

1. Harinesan N, Silsby M, Simon NG. Carpal tunnel syndrome. *Handb Clin Neurol*. 2024;201:61-88. doi: 10.1016/B978-0-323-90108-6.00005-3. PMID: 38697747.
2. Demircay E, Civelek E, Cansever T, Kabatas S, Yilmaz C. Anatomic variations of the median nerve

in the carpal tunnel: a brief review of the literature. *Turk Neurosurg*. 2011;21(3):388-96. doi: 10.5137/1019-5149.JTN.3073-10.1. PMID: 21845577.

3. Verdugo RJ, Salinas RS, Castillo J, Cea JG. Surgical versus non-surgical treatment for carpal tunnel syndrome. *Cochrane Database Syst Rev*. 2002;(2): CD001552. doi: 10.1002/14651858.CD001552. Update in: *Cochrane Database Syst Rev*. 2003;(3):CD001552. PMID: 12076416.

4. Jarvik JG, Comstock BA, Kliot M, Turner JA, Chan L, Heagerty PJ, Hollingworth W, Kerrigan CL, Deyo RA. Surgery versus non-surgical therapy for carpal tunnel syndrome: a randomised parallel-group trial. *Lancet*. 2009 Sep 26;374(9695):1074-81. doi: 10.1016/S0140-6736(09)61517-8. PMID: 19782873.

5. Lanz U. Anatomical variations of the median nerve in the carpal tunnel. *J Hand Surg Am*. 1977 Jan;2(1):44-53. doi: 10.1016/s0363-5023(77)80009-9. PMID: 839054.

6. Henry BM, Zwinczewska H, Roy J, Vikse J,

- Ramakrishnan PK, Walocha JA, Tomaszewski KA. The Prevalence of Anatomical Variations of the Median Nerve in the Carpal Tunnel: A Systematic Review and Meta-Analysis. *PLoS One*. 2015 Aug 25;10(8):e0136477. doi: 10.1371/journal.pone.0136477. Erratum in: *PLoS One*. 2015;10(9):e0138300. PMID: 26305098; PMCID: PMC4549253.
7. Demircay E, Civelek E, Cansever T, Kabatas S, Yilmaz C. Anatomic variations of the median nerve in the carpal tunnel: a brief review of the literature. *Turk Neurosurg*. 2011;21(3):388-96. doi: 10.5137/1019-5149.JTN.3073-10.1. PMID: 21845577.
8. Wynter S, Dissabandara L. A comprehensive review of motor innervation of the hand: variations and clinical significance. *Surg Radiol Anat*. 2018 Mar;40(3):259-269. doi: 10.1007/s00276-017-1898-8. Epub 2017 Jul 19. PMID: 28725918.
9. Smith JL, Siddiqui SA, Ebraheim NA. Comprehensive Summary of Anastomoses between the Median and Ulnar Nerves in the Forearm and Hand. *J Hand Microsurg*. 2019 Apr;11(1):1-5. doi: 10.1055/s-0038-1672335. Epub 2018 Oct 29. PMID: 30911205; PMCID: PMC6431281.
10. Kim BJ, Date ES, Lee SH, Lau EW, Park MK. Unilateral all ulnar hand including sensory without forearm communication. *Am J Phys Med Rehabil*. 2004 Jul;83(7):569-73. doi: 10.1097/01.phm.0000130034.76547.4f. PMID: 15213484.
11. Caetano EB, Vieira LA, Sabongi Neto JJ, Caetano MF, Sabongi RG. Riché-Cannieu Anastomosis: Structure, Function, and Clinical Significance. *Rev Bras Ortop (Sao Paulo)*. 2019 Sep;54(5):564-571. doi: 10.1016/j.rbo.2017.12.019. Epub 2019 Oct 29. PMID: 31736524; PMCID: PMC6855928.
12. Roy J, Henry BM, Pękala PA, Vikse J, Saganiak K, Walocha JA, Tomaszewski KA. Median and ulnar nerve anastomoses in the upper limb: A meta-analysis. *Muscle Nerve*. 2016 Jun;54(1):36-47. doi: 10.1002/mus.24993. Epub 2016 Apr 27. PMID: 26599506.

Acute abdomen due to spontaneous rupture of adrenal gland metastasis as initial clinical manifestation of lung cancer – a rare case presentation

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Background: Suprarenal glands are a common site for metastasis of lung cancer. These metastases are usually solitary, asymptomatic and diagnosed incidentally during staging of patients with lung cancer. Symptomatic suprarenal gland metastases at the time of initial lung cancer diagnosis are extremely rare. Our **aim** is to present a case of lung cancer with initial clinical presentation of acute abdomen caused by massive retroperitoneal hemorrhage as a result of spontaneous rupture of unilateral huge adrenal metastasis.

Case presentation: We present a 66-year old male patient with sudden onset of severe abdominal pain. Rebound tenderness and rigidity of abdomen were established on physical examination. Massive liquid collection in retroperitoneal space, tumor mass of the right suprarenal gland, free intraperitoneal liquid and tumor of the right lower lung lobe were revealed by computed tomography. Massive right-sided retroperitoneal hemorrhage, intraperitoneal blood collection and necrotic tumor of the right adrenal gland were established by laparotomy on emergency. Histological examination of specimen taken from the adrenal gland tumor revealed metastasis of squamous cell lung carcinoma **Conclusion:** Our case is unique because it presents a quite rare initial expression of metastatic lung cancer – massive retroperitoneal hemorrhage due to rupture of huge adrenal metastasis. We consider that this publication might be useful for practice.

Keywords: acute abdomen, retroperitoneal hemorrhage, adrenal metastasis, lung cancer

Introduction

Lung cancer is one of the most common types of cancers, affecting about 1 in 16 people, and continues to be one of the leading causes of death worldwide (1). The poor prognosis of lung cancer is due to its frequently delayed diagnosis, as it doesn't present with symptoms until it begins to metastasize. Lung cancer can metastasize through both the lymphatic and hematogenous routes, with the most common sites being the brain, bones, and adrenal glands (2). Very rarely, these adrenal metastases can rupture and cause massive retroperitoneal hemorrhage and acute abdomen. Adrenal gland hemorrhage is a rare but potentially fatal condition, with low incidence in post-mortem examinations (0.14%–0.8%). Symptoms typically include acute abdominal pain, nausea, lethargy, weakness, and malaise. (3) Notably, adrenal gland metastasis rupture as the first sign of metastatic lung cancer is exceptionally uncommon. While evidence

supports metastatic spread from the lungs to the adrenal glands, and the potential for bilateral adrenal hemorrhages exists, there's ongoing discussion regarding whether adrenal hemorrhage can serve as the initial manifestation of lung cancer metastasis (2). Proposed mechanisms suggest tumor expansion leading to increased vascularity, possibly mediated by various growth factors and proteases (1). The potential occurrence of intra-tumoral hemorrhage within the adrenal glands should be considered as a potential cause of acute abdomen and death (3). Acute abdomen is a surgical emergency which is a result of a wide range of etiologies including infection, obstruction, vascular occlusion, or rupture of an organ (3). It is characterized as an abdominal complication caused by peritoneal leakage leading to peritonitis or the rupture of an abdominal organ. It presents mainly with sudden severe abdominal pain within 24 hrs, along with nausea and vomiting (3).

Our aim is to present a case of lung cancer with initial clinical presentation of acute abdomen caused by massive retroperitoneal and intraperitoneal hemorrhage as a result of spontaneous rupture of large unilateral adrenal metastasis.

Case presentation

We present a 66-year old male patient with sudden onset of severe abdominal pain. On examination, the patient was hemodynamically unstable. His abdomen was moderately distended with diffuse tenderness. Plain thoraco-abdominal X-ray showed irregular tumor mass of the lower lobe of the left lung, without free air under the diaphragm (fig. 1). Ultrasonography of the abdomen revealed gross heterogeneous collection between liver and right kidney.

Contrast enhanced thoraco-abdominal tomography revealed: solid tumor mass in 7th segment of the right lung (39/42 mm), peripheral tumor mass of the left lung, necrotic tumor mass of the right suprarenal gland (36/42 mm) and the left suprarenal gland (13/24 mm), retroperitoneal, perihepatic, perisplenic and pelvic fluid collection with density of 58 to 60 Hounsfield units suggestive of hemoperitoneum and retroperitoneal hematoma (fig. 2, 3, 4).

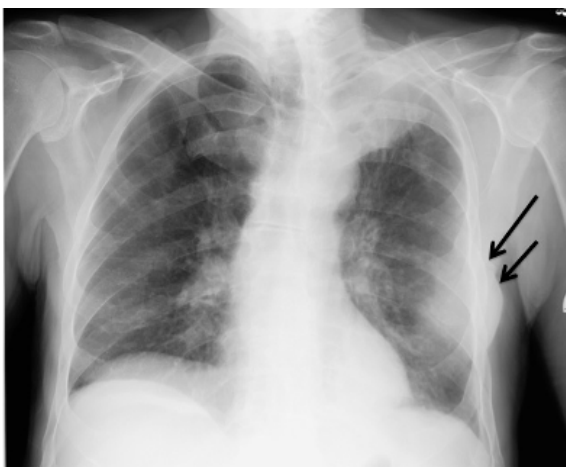


Figure 1. Plain thoraco-abdominal X-ray – peripheral tumor mass of left lung (pointed by black arrows).

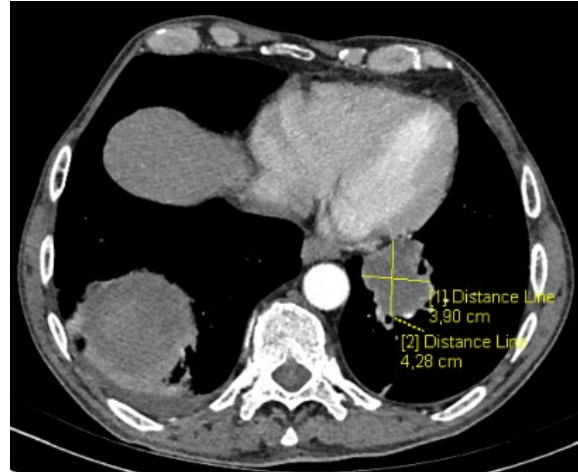


Figure 2. Contrast enhanced thoracic tomography: bilateral lung tumors.

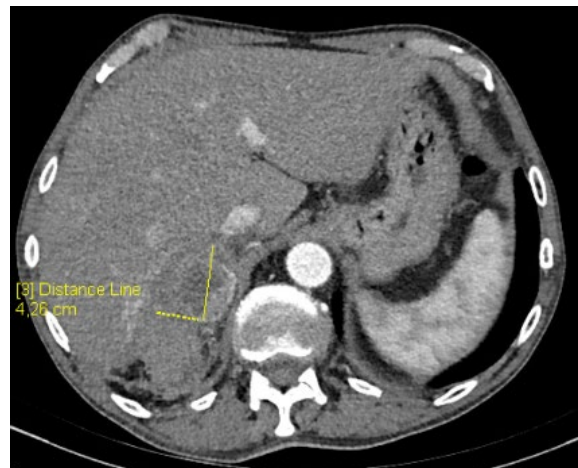


Figure 3. Contrast enhanced abdominal tomography: necrotic tumor mass of the right suprarenal gland.



Figure 4. Contrast enhanced abdominal tomography revealed retroperitoneal fluid collection (circled in red).

Exploratory laparotomy was done after optimizing the patient's condition. Massive right-sided retroperitoneal hemorrhage, intraperitoneal blood collection and necrotic tumor of the right adrenal gland were established. The right adrenal gland with the tumor mass was removed. Histological examination of specimens taken from the adrenal gland tumor revealed metastasis of squamous cell lung carcinoma.

Discussion

Lung cancer spread to the adrenal glands is known as adrenal metastasis. Lung cancer that spreads to the adrenals or any other area of the body is considered stage 4, which is advanced. The adrenal glands are the fourth most common site of metastatic involvement (following lungs, liver, and bone). Adrenal metastases are fairly common and present in 3% to 7% of people at the time of their primary cancer diagnosis (6). On rare occasions, adrenal metastases are the first sign of lung cancer. Most of the time when cancer spreads to the adrenal glands there are no symptoms. In fact, in one study, only 5% of people with adrenal metastases had symptoms (7).

Adrenal metastases do not usually result in adrenal insufficiency unless significant portions of both adrenals are affected. Patients with adrenal metastases may have localized symptoms like back pain or abdominal pain due to tumor mass effect if the tumor is large or rapidly growing. This could indicate local invasion or retroperitoneal hemorrhage. Clinical manifestations can range from subclinical unilateral hemorrhage to massive bilateral hemorrhage leading to cardiovascular collapse and death. Possible etiologies include anticoagulation, sepsis, bleeding disorders, metastatic disease, or trauma (8). Among metastatic

diseases, the lung, GI tract, breast, and kidney are the most common primary tumors.

We present a case of acute abdomen as the first sign of advanced lung cancer. This state of acute abdomen was due to spontaneous intraperitoneal and retroperitoneal hemorrhage. Spontaneous hemoperitoneum is a rare and life-threatening condition. The most common etiologies are gynecologic, splenic, and hepatic, abdominal vasculature without preceding trauma. Spontaneous retroperitoneal bleeding is also a rare disease that presents mainly with acute abdominal pain and shock that may be fatal when it occurs. There are several well-recognized causes of retroperitoneal bleeding such as ruptured aortic aneurysm, retroperitoneal neoplasms, and coagulopathy. Our case of advanced lung cancer presents an extremely rare clinical picture of acute abdomen as a result of two life-threatening conditions: intraperitoneal hemorrhage and retroperitoneal hematoma. These life-threatening conditions were the result of necrosis of a large metastasis of the suprarenal gland with ruptured peritoneal sheet with bleeding into the peritoneal cavity.

Conclusion

Our case is unique as it describes a rare initial presentation of metastatic lung cancer with a massive retroperitoneal and intraperitoneal hemorrhage, due to the rupture of large adrenal metastasis. The incidence of adrenal metastasis being the initial presentation of primary lung cancer is extremely low, and symptomatic metastases with hemorrhage can be found only in a few cases reported in the literature. We consider this publication to be useful for practice and with this case report we aim to increase awareness of this rare presentation among clinicians.

References

1. “Lung Cancer Statistics: How Common Is Lung Cancer?” *Lung Cancer Statistics | How Common Is Lung Cancer?* | American Cancer Society, www.cancer.org/cancer/types/lung-cancer/about/key-statistics.html#:~:text=Overall%2C%20the%20chance%20that%20a,t%2C%20the%20risk%20is%20lower. Accessed 13 Apr. 2024.
2. Popper, Helmut H. “Progression and Metastasis of Lung Cancer.” *Cancer Metastasis Reviews*, U.S. National Library of Medicine, Mar. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4821869/#:~:text=Another%20enigma%20is%20the%20selection,squamous%20cell%20carcinomas%20prefer%20bones.
3. Mehmood, Khawar T. “Adrenal Hemorrhage.” *StatPearls [Internet]*., U.S. National Library of Medicine, 20 Mar. 2023, www.ncbi.nlm.nih.gov/books/NBK555911/.
4. Patterson, John W. “Acute Abdomen.” *StatPearls [Internet]*., U.S. National Library of Medicine, 10 July 2023, www.ncbi.nlm.nih.gov/books/NBK459328/.
5. Guérin, E, et al. “Acute Abdomen: A Rare Presentation of Lung Cancer Metastasis.” *Case Reports in Medicine*, U.S. National Library of Medicine, 2009, www.ncbi.nlm.nih.gov/pmc/articles/PMC2762241/.
6. Bazhenova, Lyudmila, et al. *Adrenal Metastases in Lung Cancer*, Journal of Thoracic Oncology, Apr. 2014
7. Cingam, Shashank R. “Adrenal Metastasis.” *StatPearls [Internet]*., U.S. National Library of Medicine, 15 Jan. 2023, www.ncbi.nlm.nih.gov/books/NBK441879/.
8. Arivazhagan, Sowbharnika, et al. “Acute Adrenal Hemorrhage as the Initial Manifestation of Metastatic Lung Adenocarcinoma: A Cautionary Tale.” *Case Reports in Oncological Medicine*, U.S. National Library of Medicine, 25 Oct. 2022, www.ncbi.nlm.nih.gov/pmc/articles/PMC9626225/.

Advantages and disadvantages of hormonal intrauterine devices – literature review

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Introduction: In 1976, the FDA approved the manufacture of the first progestin-releasing intrauterine system in the United States. The second intrauterine system was approved in 1990 and has since been used in more than 120 countries around the world. Nowadays, a number of publications have been accumulated on the contraceptive effect of the devices and on their non-contraceptive benefits related to the antiproliferative action on the endometrium, which determine the main advantages of the pessary. Other studies focus on the unwanted effects of hormonal intrauterine devices. **Aim:** The aim of this study is to evaluate the literature and present a review about the advantages and disadvantages, indications and contraindications of levonorgestrel-releasing intrauterine systems. **Resources and methods:** A literature search of PubMed data base was conducted. Used key-words were: hormonal intrauterine devices; levonorgestrel-releasing intrauterine system. **Results and discussion:** More than 190 publications were found for the last 3 years, which reflect the interest of researchers over the years and the relevance of the problem today. **Conclusion:** The review of the literature on the subject provides up-to-date information from the reviewed studies on the advantages of levonorgestrel-releasing intrauterine pessaries and the extended indications for their use in gynecological practice. New publications related to the side effects of intrauterine systems are also indicated.

Keywords: hormonal intrauterine devices; levonorgestrel-releasing intrauterine system

Introduction

The intrauterine device is a long-acting reversible type of contraception. It has been used since 1976, when the FDA approved the manufacture of the first progestin-releasing intrauterine system in the United States. This system is indicated for 1-year contraception in parous women.

The levonorgestrel-releasing intrauterine systems – named Mirena, Liletta and Skyla are FDA approved in USA. Mirena was approved first in Finland in 1990 and in the United States in 2000. Since then it has been used in more than 120 countries around the world to prevent unintended pregnancy. Mirena contains 52 mg of levonorgestrel which release rate is 20 µg per day and provides contraception for up to 5 years.

A number of publications have been accumulated on the contraceptive effect of the devices and on their non-contraceptive benefits related to the antiproliferative action on the endometrium. Nowadays, LNG-IUS can

be used for treatment of heavy menstrual bleeding, endometriosis, adenomyosis, fibroids, or endometrial hyperplasia, which is the main advantage of the pessary. Other studies focus on the unwanted effects of hormonal intrauterine devices – breakthrough bleeding for the first three to nine months after insertion.

Aim

The aim of this study is to evaluate the literature and present a review about the advantages and disadvantages, indications and contraindications of levonorgestrel-releasing intrauterine systems.

Resources and methods

A literature search was conducted in the PubMed database. Keywords used are: hormonal intrauterine devices; levonorgestrel-releasing intrauterine system. More than 190 publications were found for the last 3 years, which reflect the interest of researchers over

the years and the relevance of the problem today. An analysis was performed for the period 2019–2023 by filtering 36 open access publications, of which 35 were included in the literature review.

Discussion

Modern levonorgestrel-releasing intrauterine pessary contraceptive methods offer high contraceptive efficacy and cost-effectiveness, and provide benefits for women and health care systems in general. The benefits of LNG-IUDs are wide-ranging on society, including reducing the need for abortion, reducing the number of completed surgical sterilization interventions, as well as reducing the number of hysterectomies and waiting times for surgical examinations [1].

According to data from Brandão ER. The Brazilian Federation of Gynecology and Obstetrics Associations has called for the levonorgestrel-releasing intrauterine system (LNG-IUS) to be introduced as a method of long-acting reversible contraception among young women [2].

Levonorgestrel intrauterine devices (LNG-IUDs) are polydimethylsiloxane (PDMS)-based non-biodegradable complex drug-device combination products with a large amount of LNG (eg 52 mg in Mirena and Liletta) to maintain long-acting local effect and extremely long duration of release in a predictable manner [3, 4, 5].

A leading mechanism of action of intrauterine devices (IUDs) determining their contraceptive effect is the foreign body-type reaction in the endometrium. In addition, there are local progestogenic effects in the uterine cavity, which are associated with pseudo-decidualization of the endometrium and glandular atrophy. Studies suggest several mechanisms of contraception – thickening of the cervical mucus, preventing the passage of sperm into the uterus, inhibiting the

capacitation or survival of sperm, and altering the endometrium, as well as the motility of the fallopian tubes [3, 6].

The levonorgestrel-releasing intrauterine system (LNG-IUS) is a first-line treatment for fertility preservation in young women with endometrial hyperplasia and should be used because of a higher rate of disease regression with fewer adverse effects compared to oral progestogens [7].

Mittermeier T. et al. analyzed and compared the LNG-IUS with non-intrauterine progestogens, placebo, surgery or no treatment, in women with histologically confirmed endometrial hyperplasia with or without atypicity. Moderate-quality evidence shows that treatment of endometrial hyperplasia up to 6 months and up to 2 years with levonorgestrel – intrauterine means is more effective than non-intrauterine progestogens. Very low-quality to low-quality evidence suggests that the LNG-IUS may reduce the risk of hysterectomy [8].

Silver S. et al. published an analysis of 13 randomized controlled trials from seven different countries regarding the regression of endometrial hyperplasia. In it, treatment of endometrial hyperplasia with the LNG-IUD was compared with systemic progesterone or no treatment. This meta-analysis states that LNG-IUS is an alternative to hysterectomy in patients who wish to preserve fertility but are diagnosed endometrial intraepithelial neoplasia or endometrial hyperplasia [9].

Cases of progression of hyperplasia to endometrioid carcinoma of the endometrium have also been described in the literature. Peng H. et al. published two such clinical cases in women of childbearing age in which progression was associated with progestin resistance [10].

Mandelbaum RS. et al. studied the effect of levonorgestrel-releasing intrauterine systems in obese women with an average BMI

of 40 kg/m² and atypical hyperplasia of the endometrium. According to him, local therapy is more effective compared to the systemic use of progestins [11].

Chen J. et al. evaluated the efficacy and safety of a gonadotropin-releasing hormone agonist (GnRHa) in combination with a levonorgestrel-releasing intrauterine device (LNG-IUD) in women with atypical endometrial hyperplasia who wished to preserve fertility. They highlighted the favorable oncological and reproductive outcomes with this type of combination [12]. Administration of LNG-IUS in patients with early-stage endometrial carcinoma can reduce adverse effects caused by long-term high-dose oral progesterone therapy according to Zhao X-L [13].

Another common pathology in women of reproductive age and premenopause are uterine fibroids. Their leading symptom is abnormal uterine bleeding leading to iron deficiency anemia. Sangkomkamhang US. et al. reported very low-quality evidence regarding LNG-IUS and the reduction of abnormal uterine bleeding or hemoglobin elevation in premenopausal women with uterine fibroids compared with oral hormone therapy [14].

Heavy menstrual bleeding is defined as blood loss of 80 ml or more per menstrual cycle, which impairs a woman's quality of life. Users of the levonorgestrel-releasing intrauterine system (LNG-IUS) report up to 90% reduction in menstrual blood loss. According to a study by Rodriguez B., LNG-IUS improve the quality of life of women with heavy menstrual bleeding [15, 16].

The National Institutes of Health and Care Excellence Guidelines for HMB recommends Levonorgestrel-Releasing Intrauterine System – Mirena as the first-line treatment for heavy uterine bleeding. Beelen P. et al. published the results of a multicenter, randomized controlled trial in the

Netherlands among women with heavy menstrual bleeding. They compared two groups of women treated with endometrial ablation and with a levonorgestrel-releasing intrauterine system. The results showed that there was no significant difference in quality of life and patient satisfaction related with a reduction in menstrual blood loss [17]. According to a study by Lethaby A., COCP is less effective than LNG IUS for the treatment of heavy menstrual bleeding [18].

HMB has a wide-ranging and debilitating effect on the lives of women with this type of pathology. According to Dutton B. From the ECLIPSE study, women usually delay seeking help even for years [19]. The ECLIPSE trial is a 10-year prospective observational cohort study in primary care among women with heavy menstrual bleeding using a hormone-releasing intrauterine system and oral therapy. Quality of life was higher than before treatment without much difference in the method of therapy used [20].

Ji L. et al. investigated the effect of levonorgestrel-releasing intrauterine pessaries in women with PCOS. They found that the mean ovarian volume was significantly smaller than the volume before LNG-IUS placement as well as an improved lipid profile compared to the control group [21].

Sergison JE. et al. found that slightly more than 20% of women using the LNG-IUS were amenorrhoeic by the first year after insertion [22].

A long-term complication of cesarean delivery is the isthmocele. This cicatrix defect from the hysterotomy suffered is associated with postmenstrual bleeding and pelvic pain. The randomized cohort study by Zhang J. et al. found that the levonorgestrel intrauterine system reduces spotting after a 9-month period as well as pelvic pain after a 3-month period [23].

Adenomyosis is an internal genital form

of endometriosis with the highest incidence among patients aged 41 to 45 years. The levonorgestrel-releasing intrauterine (LNG-IUS) system is an effective primary treatment for adenomyosis. The leading disadvantage is spontaneous expulsion of the pessary. Yang H, et al. performed a study and found a low rate of spontaneous expulsion in five years of use. This established the LNG-IUS as a safe method and an effective method to reduce heavy menstrual bleeding [24, 25].

Zhu L. et al. propose a minimally invasive hysteroscopic method to prevent expulsion of the intrauterine pessary in women with adenomyosis and heavy menstrual bleeding. Their study showed that by hysteroscopic cold knife surgery system (HCSS) the LNG-IUD was fixed and this prevented the risk of expulsion. In the study by Armstrong MA. breastfeeding is associated with a lower risk of expulsion, and placing a pessary until the third day after birth with a high risk. The optimal period is more than 6 weeks after birth according to the authors [26, 27].

An analysis by Abbas AM et al. showed that the LNG-IUS reduced symptoms such as dysmenorrhea and heavy menstrual bleeding, while also reducing the thickness of the endometrium, which led to improved laboratory results [28].

Another study highlighted the role of dienogest and the levonorgestrel intrauterine system as agents that pelvic pain and abnormal bleeding in women with adenomyosis [29].

Another team of authors studied and compared different methods of therapy in women with endometriosis, and the result of their work was that LNG-IUS in combination with HIFU combined and GnRH was more effective than both methods separately or in combination [30].

Another disadvantage of intrauterine pessaries is related to the dose of levonorgestrel. It must be high for the purpose of contraception, but also low enough to avoid the systemic effects of progestagens. The relationship between the use of intrauterine systems with levonorgestrel containing 52, 19.5 and 13.5 mg of the hormone and ectopic pregnancy in cohort study of Meaidi A. et al. The risk of ectopic pregnancy is increased with levonorgestrel IUDs at a dose of 13.5 mg compared to other types of hormonal contraceptives [31].

Another disadvantage is the conflicting data from studies, according to which LNG-IUS users are at increased risk of developing breast cancer, especially postmenopausal women and with longer use [32]. Conz L. et al. performed a systematic review and meta-analysis of the literature regarding the 52 mg LNG-IUS and breast cancer [32]. The analysis showed that the LNG-IUS increased the risk of breast cancer regardless of age and indication, with the risk of breast cancer being greater in older women with risk factors for breast cancer [33].

Other authors have investigated the benefits of the LNG-IUS in preventing the development of endometrial pathology in women receiving tamoxifen as adjuvant therapy for breast cancer. An advantage of the LNG-IUS is that it reduces the incidence of endometrial polyps and endometrial hyperplasia in women with breast cancer taking tamoxifen, but a disadvantage is an increased risk of abnormal vaginal bleeding or spotting after one to two years [34].

Scarce bleeding – spotting during the first two years after insertion is indicated as a drawback and a reason for discontinuing the application before the fifth year in women using the pessary with levonorgestrel for non-contraceptive purposes [35].

Conclusion

The review of the literature on the subject provides up-to-date information from the reviewed studies on the advantages of levonorgestrel-releasing intrauterine pessaries and the extended indications for their use in gynecological practice. New publications related to the side effects of intrauterine systems are also indicated.

References

1. Gemzell-Danielsson K, Kubba A, Caetano C, Faustmann T, Lukkari-Lax E, Heikinheimo O. More than just contraception: the impact of the levonorgestrel-releasing intrauterine system on public health over 30 years. *BMJ Sex Reprod Health*. 2021;47(3):228-230.
2. Brandão ER. Métodos contraceptivos reversíveis de longa duração no Sistema Único de Saúde: o debate sobre a (in)disciplina da mulher [Long-acting reversible contraception methods in the Brazilian Unified National Health System: the debate on women's (in)discipline. *Cien Saude Colet* [Internet]. 2019;24(3):875-9.
3. Bao Q, Fanse S, Lu XJ, Burgess DJ. Impact of drug loading on release from levonorgestrel intrauterine systems. *Int J Pharm* [Internet]. 2023;631(122532):122532.
4. Bao Q, Zou Y, Wang Y, Kozak D, Choi S, Burgess DJ. Drug release testing of long-acting intrauterine systems. *J Control Release* [Internet]. 2019;316:349-58.
5. Jensen JT, Reinecke I, Post TM, Lukkari-Lax E, Hofmann BM. Extended use of levonorgestrel-releasing intrauterine system (LNG-IUS) 52 mg: A population pharmacokinetic approach to estimate in vivo levonorgestrel release rates and systemic exposure including comparison with two other LNG-IUSs. *Contraception* [Internet]. 2023;120(109954):109954.
6. Smith-McCune K, Thomas R, Averbach S, Seidman D, Takeda M, Houshdaran S, et al. Differential effects of the hormonal and copper intrauterine device on the endometrial transcriptome. *Sci Rep* [Internet]. 2020;10(1):6888.
7. Kim MK, Seong SJ, Park DC, Hong JH, Roh JW, Kang SB. Comparison of diagnostic accuracy between endometrial curettage and aspiration biopsy in patients treated with progestin for endometrial hyperplasia: a Korean Gynecologic Oncology Group study. *J Gynecol Oncol* [Internet]. 2020;31(4):e51.
8. Mittermeier T, Farrant C, Wise MR. Levonorgestrel-releasing intrauterine system for endometrial hyperplasia. *Cochrane Database Syst Rev* [Internet]. 2020;9(9):CD012658.
9. Silver S, Arnold JJ. Levonorgestrel-releasing intrauterine system for regression of endometrial hyperplasia. *Am Fam Physician*. 2021;104(1):26-7.
10. Peng H, Jiang J, Li X. Endometrial cancer following levonorgestrel-releasing intrauterine system insertion in young women with atypical hyperplasia: Two case reports and literature review. *Reprod Sci* [Internet]. 2022;29(11):3278-84.
11. Mandelbaum RS, Ciccone MA, Nusbaum DJ, Khoshchehreh M, Purswani H, Morocco EB, et al. Progestin therapy for obese women with complex atypical hyperplasia: levonorgestrel-releasing intrauterine device vs systemic therapy. *Am J Obstet Gynecol* [Internet]. 2020;223(1):103.e1-103.e13.
12. Chen J, Cao D, Yang J, Yu M, Zhou H, Cheng N, et al. Oncological and reproductive outcomes for gonadotropin-releasing hormone agonist combined with aromatase inhibitors or levonorgestrel-releasing intrauterine system in women with endometrial cancer or atypical endometrial hyperplasia. *Int J Gynecol Cancer* [Internet]. 2022;32(12):1561-7.
13. Zhao X-L, Du Z-Q, Zhang X, Yao Z, Liang Y-Q, Zhao S-F. Fertility-preserving treatment in patients with early-stage endometrial cancer: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)* [Internet]. 2021;100(48):e27961.
14. Sangkomkarnhang US, Lumbiganon P, Pattanittum P. Progestogens or progestogen-releasing intrauterine systems for uterine fibroids (other than pre-operative medical therapy). *Cochrane Database Syst Rev* [Internet]. 2020;11(11):CD008994.
15. Rodriguez B, Lethaby M, Jordan A. Progestogen-releasing intrauterine systems for heavy menstrual bleeding. *Cochrane Database Syst Rev* [Internet]. 2020;6(6).
16. Rodriguez B, Lethaby M, Low A, Cameron C. Cyclical progestogens for heavy menstrual bleeding. *Cochrane Database Syst Rev* [Internet]. 2019;8(8).
17. Beelen P, van den Brink MJ, Herman MC, et al. Levonorgestrel-releasing intrauterine system versus endometrial ablation for heavy menstrual bleeding. *Am J Obstet Gynecol* [Internet]. 2021;224(2):187.e1-187.e10.
18. Lethaby A, Wise MR, Weterings MA, Bofill Rodriguez M, Brown J. Combined hormonal contraceptives for heavy menstrual bleeding. *Cochrane Database Syst Rev* [Internet]. 2019;2(2):CD000154.
19. Dutton B, Kai J. Women's experiences of heavy menstrual bleeding and medical treatment: a qualitative study in primary care. *Br J Gen Pract* [Internet]. 2023;73(729):e294-301.
20. Kai J, Dutton B, Vinogradova Y, Hilken N, Gupta J, Daniels J. Rates of medical or surgical treatment for women with heavy menstrual bleeding: the ECLIPSE trial 10-year observational follow-up study. *Health Technol Assess* [Internet]. 2023;27(17):1-50.
21. Ji L, Jin L, Shao MJ, Hu M, Xu J. Levonorgestrel-releasing intrauterine system effects on metabolic variables in PCOS. *Gynecol Endocrinol*. 2023;39(1):2208667. doi:10.1080/09513590.2023.2208667
22. Sergison JE, Maldonado LY, Gao X, Hubacher D. Levonorgestrel intrauterine system associated amenorrhea: a systematic review and metaanalysis. *Am J Obstet Gynecol* [Internet]. 2019;220(5):440-448.e8.

23. Zhang J, Zhu C, Yan L, Wang Y, Zhu Q, He C, et al. Comparing levonorgestrel intrauterine system with hysteroscopic niche resection in women with postmenstrual spotting related to a niche in the uterine cesarean scar: a randomized, open-label, controlled trial. *Am J Obstet Gynecol* [Internet]. 2023;228(6): 24. 712.e1-712.e16
25. Schrager S, Yogendran L, Marquez CM, Sadowski EA. Adenomyosis: Diagnosis and management. *Am Fam Physician*. 2022;105(1):33–8.
26. Yang H, Wang S, Fu X, Lan R, Gong H. Effect of modified levonorgestrel-releasing intrauterine system in human adenomyosis with heavy menstrual bleeding. *J Obstet Gynaecol Res* [Internet]. 2022;48(1): 161–8. Available from: <http://dx.doi.org/10.1111/jog.15031>
27. Zhu L, Yang X, Cao B, Tang S, Tong J. The suture fixation of levonorgestrel-releasing intrauterine device using the hysteroscopic cold-knife surgery system: an original method in treatment of adenomyosis. *Fertil Steril* [Internet]. 2021;116(4):1191–3. Available from: <http://dx.doi.org/10.1016/j.fertnstert.2021.05.113>
28. Armstrong MA, Raine-Bennett T, Reed SD, Gatz J, Getahun D, Schoendorf J, et al. Association of the timing of postpartum intrauterine device insertion and breastfeeding with risks of intrauterine device expulsion. *JAMA Netw Open* [Internet]. 2022;5(2):e2148474.
29. Abbas AM, Samy A, Atwa K, Ghoneim HM, Lotfy M, Saber Mohammed H, et al. The role of levonorgestrel intra-uterine system in the management of adenomyosis: A systematic review and meta-analysis of prospective studies. *Acta Obstet Gynecol Scand* [Internet]. 2020;99(5):571–81.
30. Benetti-Pinto CL, Mira TAA, Yela DA, Teatin-Juliato CR, Brito LGO. Pharmacological Treatment for Symptomatic Adenomyosis: A Systematic Review. *Tratamento farmacológico para adenomiose sintomática: revisão sistemática*. *Rev Bras Ginecol Obstet*. 2019;41(9):564-574. doi:10.1055/s-0039-1695737
31. Ogtontuya A, Jeng C-J, Wu T-N, Chuang LT, Shen J. Comparison of the treatment efficacies of HIFU, HIFU combined with GnRH-a, and HIFU combined with GnRH-a and LNG-IUS for adenomyosis: A systematic review and meta-analysis. *Taiwan J Obstet Gynecol* [Internet]. 2023;62(2):226–38.
32. Meaidi A, Torp-Pedersen C, Lidegaard Ø, Mørch LS. Ectopic pregnancy risk in users of levonorgestrel-releasing intrauterine systems with 52, 19.5, and 13.5 mg of hormone. *JAMA* [Internet]. 2023;329(11):935–7.
33. Zürcher A, Knabben L, Janka H, Stute P. Influence of the levonorgestrel-releasing intrauterine system on the risk of breast cancer: a systematic review. *Arch Gynecol Obstet* [Internet]. 2023;307(6):1747–61.
34. Conz L, Mota BS, Bahamondes L, Teixeira Dória M, Françoise Mauricette Derchain S, Rieira R, et al. Levonorgestrel-releasing intrauterine system and breast cancer risk: A systematic review and meta-analysis. *Acta Obstet Gynecol Scand* [Internet]. 2020;99(8):970–82.
35. Romero SA, Young K, Hickey M, Su HI. Levonorgestrel intrauterine system for endometrial protection in women with breast cancer on adjuvant tamoxifen. *Cochrane Database Syst Rev* [Internet]. 2020;12(2):CD007245.
36. Miranda L, Sousa MH, Faundes A, Juliato C. Continuation Rates of the 52-mg Levonorgestrel-releasing Intrauterine System according to the Primary Reason for its Use. *Taxas de continuação com o sistema intrauterino de liberação de levonorgestrel de 52 mg de acordo com o motivo principal de seu uso*. *Rev Bras Ginecol Obstet* [Internet]. 2021;43(4):291–6.

Pediatric orbital tumors: an illustrative case report with a review of differential diagnosis

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Orbital tumors are relatively rare in the pediatric population and are among the most undesirable causes of childhood proptosis. This report aims at presenting the case of an infant with unilateral proptosis, followed by a concise review of the differentially diagnostic aspects of pathological orbital masses in children, highlighting their diversity and the importance of their timely diagnosis. The patient is a female, the first of twins, born at 37 weeks of gestation with a body weight of 2160 g. Neonatal O2 therapy was applied due to RDS. She first presented to the University Eye Clinic at the age of 5 weeks, due to proptosis of the right eye noticed by her parents. Eyeball motility, as well as all structural and functional aspects of the eye, were unaffected. Contrast CT showed a lobulated, contrast-enhancing formation in the right retrobulbar space, ~16 mm axially, enveloping the optic nerve, engaging the extraocular musculature, and pushing the bulb ventrolaterally, with contrast-enhanced MRI reaffirming the finding. A biopsy (ideally excisional, if possible) was recommended, but parents have insofar refused. Considering the extreme diversity of orbital mass-occupying lesions, their forms can range from clinically and pathologically benign entities, such as hemangiomas and dermoid cysts, to extremely aggressive rhabdomyosarcomas, neuroblastomas and Ewing's sarcoma metastases, among numerous others. This report serves as a demonstrative case showcasing the extremely broad differential diagnosis of pediatric orbital tumors and as an educational resource on said topic.

Keywords: *pediatric, orbital tumor, differential diagnosis*

Introduction

Unilateral proptosis in the pediatric population is an uncommon condition, yet the diversity of its potential etiologies is extreme, ranging from mundane infections to sarcomas. We would like to use this case report to illustrate the complexity regarding the diagnosis, treatment and prognosis a medical professional may encounter when dealing with such pathologies.

The case

The patient is a female, born first of twins at late preterm (37 GW) with low birth weight (2160 g) and IRDS, necessitating oxygen therapy for 6 days. At 5 weeks of age, she presented to the Plovdiv Medical University Eye Clinic due to “bulging” of the right eye noticed by her parents. Her twin brother had no signs of ocular pathology. On ophthalmological assessment, inspection revealed a unilateral axial proptosis and a small elevated perilimbic lesion of unknown etiology in the right eye. Slit lamp examination showed bilaterally clear corneas, anterior chambers, lens, vitreous bodies and

regularly shaped irises. Fundoscopy revealed healthy optic discs, on the retinal level and normal maculas and vascularization bilaterally. Both pupils were round and centered and reacted adequately to light directly and consensually, indicating a lack of damage or compression of the optic nerve. Eyeball motility was not restricted, pointing to normal anatomophysiologic functioning of extraocular musculature.

On imaging, contrast enhanced CT and MRI showed a contrast enhancing lobulated mass in the retrobulbar space, ~16 mm axially. It enveloped the optic nerve and engaged the extraocular musculature. Excisional biopsy was suggested, but refused by the parents.

5 months after the initial consultation, the patient was brought for a control examination. The degree of proptosis was shown to be significantly reduced, the perilimbic lesion-still present, with all other parameters of assessment remaining normal. As of the time of writing, no repeat imaging nor biopsy has been performed, therefore this case remains open and observation is to be maintained.

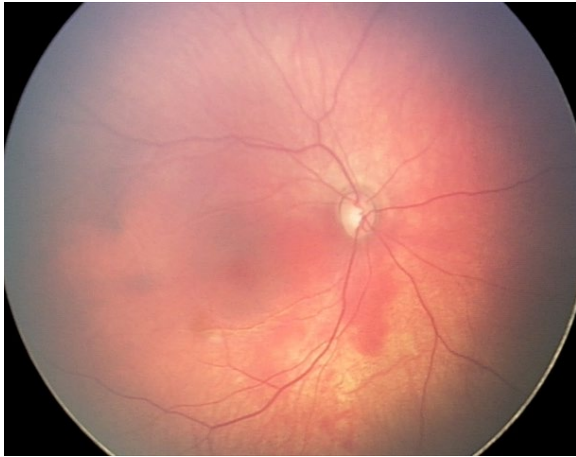


Figure 1. Fundoscopy of the right eye, showcasing healthy optic discs, macula and vascularization

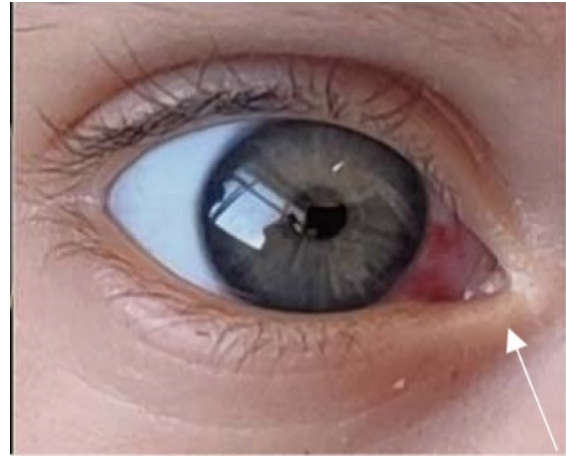


Figure 2. Small elevated red perilimbal lesion

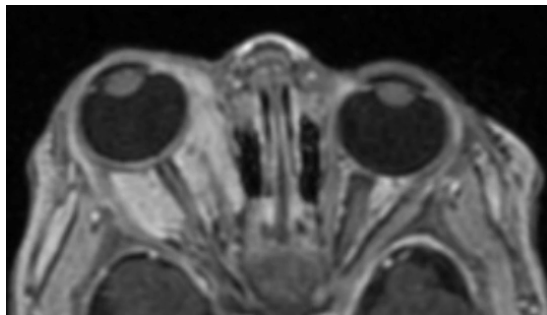


Figure 3 and 4. Axial and Coronal CE MRI, showcasing the contrast enhancing mass and its relationship to some retrobulbar structures



Figure 5. A significantly reduced proptosis at month 5

Discussion

Without histologic confirmation, theoretically no pathological entities can be excluded from the potential differential diagnosis. We would thus like to concisely present some of the more common causes of unilateral proptosis in the pediatric population.

The most common cause of this condition

is orbital cellulitis (1) – an infection of the retrobulbar soft tissue, which usually presents with general and local signs of inflammation, the latter consisting primarily of oedema and erythema of the eyelids (2). The inflammation may also be compartmentalized to the retrobulbar space, which could result in a proptosis without local inflammatory signs (3).

Orbital dermoid cysts are the most common orbital masses in children (4). They're congenital choriostomas (histologically normal tissue in an abnormal location), enclosed in a fibrous sac lined with keratinized epithelium (5). The majority of these lesions are superficial and present with a periocular bulge around the brow or eyelids, but deep dermoids cysts may produce an isolated proptosis. The American Academy of Ophthalmology recommends early drainage as a preventative measure, as minor trauma may result in cyst sac rupture and spillage, leading to significant orbital infections (6).

Capillary hemangiomas are the most common neoplasms per se causing unilateral proptosis in children (7). They consist of abnormally proliferated endothelium and tend to cause a rapid onset of proptosis in the first weeks of life, usually followed by involution in the following years, with 75% of cases completely resolving by year 7. Where risk of significant complications is noted, systemic and topical treatment with beta-blockers and corticosteroids are standardly used with very good results. Surgery is rarely necessary (8).

Lymphangiomas are dilatative malformations of the lymphatic vessels which can be congenital or acquired (9). Their clinical course tends to be unpredictable, as upper respiratory tract infections or minor trauma, resulting in bleeding into the mass, among others, may cause rapid and dramatic increases in size and degree of proptosis. MRI is pathognomonic for these lesions, displaying a multitude of cystic lesions with layering of serum and blood. Treatment of choice is medical (sildenafil or sirolimus) as attempts for surgical excision often result in recurrences and heavy bleeding (6).

Optic nerve gliomas are characteristically benign and slow growing in the pediatric population, unlike their adult counterparts

(glioblastoma), which are extremely aggressive. Plexiform neurofibromas are tumors of the Schwann cells of the optic nerve and share many clinical features with gliomas. The former of these entities has a ~30% rate of association with the NF1 syndrome, while the latter appears almost exclusively in its context. Characteristic for neural tumors is their tendency to extend into the cranium (2, 6).

The most common histologically malignant cause of childhood proptosis is rhabdomyosarcoma, predominantly embryonal, but the extremely malignant alveolar subvariant may also be observed (10). Rhabdomyosarcomas are undifferentiated masses of mesenchymal cells, which cause very rapidly progressing proptosis. On imaging, invasion into neighboring tissues and destruction of bone may be observed. Oedema of the eyelid and conjunctiva are common extraorbital findings. Treatment of choice is prompt surgical excision, followed by chemoradiotherapy (6).

Extraorbital tumors tend to metastasize more commonly into the orbit in children as compared to in the adults, where uveal localization is more characteristic. The most common such lesion in children is the neuroblastoma, followed by Ewing's sarcoma (2).

Potential complications of significant proptosis include exposure keratopathy – due to the eyelids' inability to fully cover the cornea (lagophthalmos), leading to evaporation of tear film and to corneal damage and potentially irreversible blindness (11). Vision loss may arise from compression and/or infiltration of the optic nerve by the mass (12, 13). Amblyopia may result from strabismus – due to non-axial displacement of the bulbus or from compromised motility, or from astigmatism in cases with significant ocular compression and deformation (2). Extension into the cranium – characteristic, but not exclusive for neural tumors – is another

possibility and may result in increased ICP, hypophyseal dysfunction, etc. (13). Malignant tumors may infiltrate and destroy local structures, bone and may eventually metastasize. Some lesions may hemorrhage, leading to a sharp rise in intraorbital pressure, compressing the ophthalmic artery and causing ischemia and necrosis of the optic nerve and retina-orbital compartment syndrome (6).

Conclusion

Proptosis in the pediatric population can be indicative of a very diverse of pathologies, all of which necessitate fundamentally differing and individual approaches regarding their diagnostics, management and prognosis. This symptom should therefore never be taken lightly by medical professionals and should always be holistically investigated, as a delayed diagnosis of a potentially clinically or histologically malignant lesion may result in permanent disabilities, or in some instances, may even lead to death. Although clinical evidence in our patient points towards a benign etiology (We believe a capillary hemangioma to be the most likely culprit), the refusal for biopsy means that there can be no final diagnosis and consequently, none of the aforementioned masses can be definitively excluded as causative agents of this proptosis. Therefore, this clinical case remains open at present and observation is to be maintained for the foreseeable future.

References

1. Kalantri SH, Balasubramanian S, Sneha LM, Poonam SN. Unilateral proptosis in a child-need for prompt diagnosis. *Sri Ramachandra J Health Sci* 2021; 1:34-7.
2. American Academy of Ophthalmology, Basic and Clinical Science Course, Section 06, Chapter 17, 2023-24
3. Hauser, A., & Fogarasi, S. (2010). Periorbital and Orbital Cellulitis. *Pediatrics in Review*, 31(6), 242–249. doi:10.1542/pir.31-6-242
4. Chung et al. From the Archives of the AFIP. Pediatric orbit tumors and tumorlike lesions: osseous lesions of the orbit. *Radiographics*. 2008;28 (4): 1193-214.
5. Lane, C., Ehrlich, W., Wright J. (1987). Orbital Dermoid Cyst. *Eye*, 1, 504-511
6. American Academy of Ophthalmology, Basic and Clinical Science Course, Section 07, Chapter 5, 2023-24
7. Barrett G et al., Capillary hemangioma (infantile periorcular hemangioma), *Survey of Ophthalmology*, Volume 38, Issue 5, 1994, Pages 399-426, ISSN 0039-6257
8. Thoomazet F. et al. "Efficacy of Systemic Propranolol for Severe Infantile Haemangioma of the Orbit and Eyelid: A Case Study of Eight Patients." *Br J Ophth*. 2012;96(3):370-4.
9. Miceli A, Stewart KM: Lymphangioma, *StatPearls*. Treasure Island (FL), 2019
10. Rao et al., A clinical update and radiologic review of pediatric orbital and ocular tumors. *Journal of Oncology*. 2013;(2013):1-22.
11. Mathenge W. Emergency management: exposure keratopathy. *Community Eye Health*. 2018;31(103):69. PMID: 30487689; PMCID: PMC6253321.
12. Atkins, E. J., Newman, N. J., & Biousse, V. (2011). Lesions of the optic nerve. *Neuro-Ophthalmology*, 159–184. doi:10.1016/b978-0-444-52903-9.00012-1
13. Hwang, J.-M., Cheon, J.-E., & Wang, K.-C. (2008). Visual prognosis of optic glioma. *Child's Nervous System*, 24(6), 693–698. doi:10.1007/s00381-007-0564-6

II. Biomedical Session

Objective polysomnographic parameters incompletely represent subjective improvement in patients with sleep bruxism treated with botulinum toxin – two case reports

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Introduction: Local administration of botulinum toxin A (BTX) is a novel therapeutic approach for patients with sleep bruxism, but data on its clinical efficacy and effects on various sleep parameters remains obscure. **Aim:** This report compares the objective changes in sleep structure and bruxism intensity with the subjectively reported improvement of sleep quality and daily symptoms after administration of BTX. **Materials and methods:** Two female patients, aged 45 and 28, presented with complaints of morning headache, jaw stiffness and teeth clenching. They underwent unattended, homebased polysomnography and were diagnosed with sleep bruxism. Patient one had bruxism index (BI) of 8.4/h and patient two – BI of 3.3/h. Sleep structure was mildly impaired in both with decreased REM-sleep percentage. Local injection of 30 units BTX in each masseter muscle and 20 units in each temporal muscle was applied, followed by additional polysomnographic evaluation thirty days after treatment. **Results and discussion:** Albeit both patients reported significant improvement regarding complaints and sleep quality, the objective measurement showed improvement only for patient 1 (posttreatment BI = 4.3/h). Considerable aggravation was found for the second patient with BI increasing to 4.6/h. However, sleep architecture was restored in both patients with REM stage normalization. **Conclusion:** The following report indicates that standard objective sleep measurements are insufficient to assess the positive clinical outcome in patients with sleep bruxism. Further investigation is needed to evaluate the benefits of the treatment.

Keywords: sleep, bruxism, botulinum toxin, polysomnography

Introduction

According to an international consensus obtained in 2013, bruxism is defined as a repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or by bracing or thrusting of the mandible and is specified by distinctive circadian manifestations as either sleep bruxism (SB) or awake bruxism (AB) (1).

The reported prevalence of SB ranges between 8% and 20% among the adult population with no gender differences (2). In a significant number of patients it leads to dental attrition, temporomandibular disorders (TMD), or even tooth fractures (3). The latter findings, alongside with the reported sleep fragmentation and association between SB/TMD and sleep disturbances such as secondary insomnia, sleep apnea and periodic limb movement disorder (PLMD) affect and

alter sleep quality and quantity, contributing to impaired quality of life (4–6).

Sleep bruxism's high prevalence and adverse effects determine the necessity of adequate and effective treatment options. Among the various treatment modalities, noteworthy are the occlusal splints, pharmacological agents, behavioural approaches, and intramuscular application of botulinum toxin (BTX) (7). Despite the favourable effects of BTX therapy on subjective complaints, the paucity of objective data validating the positive clinical outcomes defines the necessity of PSG studies corroborating the benefits of this treatment.

Case report presentation

We present two case reports of patients with SB and compare the objective changes in sleep structure and bruxism intensity with

the subjectively reported improvement of sleep quality after administration of BTX.

Patient 1 is a 45-year-old female with a body mass index (BMI) of 22.7 (height = 160 cm, weight = 58 kg) who reports jaw clenching and tooth grinding during her sleep and occasionally during wakefulness. This grinding and clenching have led to tooth surface loss and fractures. Pain and discomfort in the masseter area are present upon awakening, more notably on the right side. The intensity of the complaints is markedly higher after alcohol consumption. The patient denies snoring or unpleasant sensations in the lower limbs prior to falling asleep. The clinical examination revealed bilateral masseter hypertrophy and dental wear. The patient has mixed anxiety-depressive disorder.

Patient 2 is a 28-year-old female with a BMI of 19.6 (height = 163 cm, weight 52 kg) who complains of morning headaches and pain in the neck, shoulders and both temporomandibular joints upon awakening, which started five years ago. Treatment with pharmacological agents and physiotherapy was performed without any positive outcome on the symptoms. The patient reports difficulties falling asleep, with a usual sleep latency of approximately an hour or two. No medical history regarding snoring or restlessness in lower limbs prior to sleep onset exists. Marked intraoral linea alba and dental attrition were objectified on clinical examination.

Both patients were referred to a somnologist and underwent home-based, unattended audio-PSG using NOX A1 PSG systems, including 10-channel electroencephalography according to 10–20 system; electrocardiography – I lead; pulse oximetry; electromyography on m. mentalis and both masseter muscles; limb movements; respiratory effort; nasal airflow. Biocalibration for the masseter and temporalis muscles EMG

recordings included maximal voluntary clenching, submaximal contraction, and swallowing. Sleep study of patient 1 confirmed the diagnosis of SB with a bruxism index (BI) of 8.4/h (high-intensity) and revealed a concomitant sleep-related movement disorder – periodic limb movement disorder with a PLMD index of 11.4/h. Sleep architecture was mildly altered with a decreased REM sleep percentage (17.7%), potentially owing to the first-night effect and an increased arousal index of 18.3. The PSG recording of patient 2 verified the diagnosis of SB, with a bruxism index of 3.3 (low intensity). It showed similarities regarding sleep structure – a reduced REM-stage percentage (19.0%) and an increased arousal index (15.6).

Both patients received intramuscular application of BTX Bocouture™, reconstituted with sterile saline solution (10 units of BTX in 0.1 ml of solution). Using a sterile syringe, the ready-to-use BTX solution was injected deep between the muscle fibres as follows: 30 IU in each masseter muscle and 20 IU in each temporalis muscle.

Clinical re-evaluation and follow-up PSG were performed one month after the BTX injection. Both subjects reported substantial improvement and reduction in symptom intensity in terms of pain severity, frequency, and improved sleep quality. The daytime symptoms were entirely resolved, there was no fatigue in the masticatory muscles, and restored jaw movement. The only side effect reported by both patients was transient jaw-muscle weakness, which required the intake of mashed food for a short period of time. On the other hand, PSG findings did not correlate with the reported subjective improvement and were notably different for both subjects. The bruxism severity index decreased in patient 1 – 4.8/h, yet it was still within the range of high-intensity SB. It is noteworthy, however, that the BI for patient

2 increased significantly from 3.3/h before treatment up to 4.6/h post-treatment signifying increased bruxism severity and placing her in the “high intensity” group. These results are in contrast to the significant improvement reported by both patients. Nonetheless, normalization in sleep architecture was observed with an increase in REM stage percentage above 25% for both women. PLMD in patient 1 persisted with a PLMD index = 31.2. None of the remaining parameters directly or indirectly reflecting bruxism severity (bruxism to arousal index, total burst count, bursts per episode, average burst duration), showed consistent reduction after the application of BTX. The only notable difference was observed in the maximal duration of bursts in seconds, an implicit marker of intensity, which was notably lower in both patients post-treatment (for patient 1 from 12.1 s to 8.6 s and for patient 2 from 11.9 s to 5.9 s).

Discussion

Sleep bruxism is defined as repetitive phasic or tonic masticatory muscle activity during sleep and is not regarded as a disorder in otherwise healthy individuals (8). Jaw clenching and muscle activity during sleep is represented objectively via EMG rhythmic masticatory muscle activity (RMMA) (9). According to previous studies, patients with SB are categorized into two groups based on the RMMA frequency – low frequency (2–4 episodes/h) and moderate-high frequency (≥ 4 episodes/h) groups (10). The etiology and pathophysiology of SB have not been fully elucidated, undoubtedly due to the multifactorial nature of this oromotor activity. The most widely supported hypothesis considers RMMA and SB because of micro-arousals during sleep, potentially due to overactivation of the ascending reticular activating system. Therefore, efficient treatment is often challenging, and current therapeutical

options fail to adequately handle the genesis of the bruxism episodes (BE) (11).

Often, splints are the first line of treatment. Nevertheless, a mounting body of evidence suggests that occlusal splints do not reduce the intensity nor the frequency of RMMA in SB, as they only provide mechanical protection and limit the detrimental effects of SB on the dentition (5, 7). Furthermore, the presence of an intraoral appliance triggers the afferentations from the periodontal ligament mechanoreceptors and jaw elevator muscles, sending discharges to the mesencephalic trigeminal nucleus and consequently provoking RMMA (5, 11). A novel and promising therapeutic approach is the intramuscular application of botulinum toxin (BTX). This method provides chemical denervation by inhibiting the acetylcholine transmission and thus reduces the intensity of RMMA during sleep. In this manner it protects sleep architecture and decreases the negative consequences of tooth grinding and clenching on the dentition with barely any adverse side effects (12).

The scarcity of previous studies reporting altered sleep parameters shows inconsistent findings, with some being comparable to the observed in our patients decreased REM sleep percentage, while others show reduced sleep efficiency and increased arousal index (2, 13). Differences in study designs and inclusion criteria are among the factors responsible for these notable discrepancies. Noteworthy, in all the studies assessing the effectiveness of BTX on BE, there was no significant reduction in the total count of RMMA or BI. Nevertheless, BTX administration decreased the intensity of the muscle contractions, measured as the number of masticatory bursts per BE, peak amplitude, or average intensity, compared to baseline EMG activity (2, 5, 7).

The increase in BI found in patient 2 after treatment questions the reliability of the

measurement tools used to assess the subjectively reported improvement. A potential explanation could be found in the fact that SB is a highly variable condition with BE frequency depending on various factors, such as sleep stages, respiratory efforts and microarousals. One theory suggests that during a second night of PSG, the number of BE increases as a result of the so-called “second night effect” (7). The latter phenomenon contributes to the increase of N2 sleep on a second night of PSG compared to the first night. Considering the fact that BE occur predominantly during the non-REM sleep, normalization in sleep architecture might offer a potential explanation to this significant difference, observed in our case (10).

As shown in a placebo-controlled trial, BTX therapy is effective for controlling RMMA intensity but has no effect on sleep structure, with no significant differences observed for both groups pre- and post-treatment, regarding sleep stage percentage, total sleep time, or arousal index (7). Considering the data above, a conclusion can be drawn, that BTX therapy does not affect the genesis of BE, but rather eliminates the adverse consequences of RMMA, thus contributing to the subjective improvement, reported by the patients.

Conclusion

The following report indicates that standard objective sleep measurements are insufficient to assess the positive clinical outcomes in patients with sleep bruxism. Additional research is required to assess the efficacy of treatment and to objectively quantify the positive clinical outcomes, which can offer valuable insights into prognosis.

Reference

1. Lobbezoo F, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, et al. Bruxism defined and graded: An international consensus. *J Oral Rehabil*. 2013 Jan;40(1):2–4.

2. Shim YJ, Lee MK, Kato T, Park HU, Heo K, Kim ST. Effects of botulinum toxin on jaw motor events during sleep in sleep bruxism patients: A polysomnographic evaluation. *Journal of Clinical Sleep Medicine*. 2014;10(3):291–8.
3. Minakuchi H, Fujisawa M, Abe Y, Iida T, Oki K, Okura K, et al. Managements of sleep bruxism in adult: A systematic review. Vol. 58, *Japanese Dental Science Review*. Elsevier Ltd; 2022. p. 124–36.
4. Dubrovsky B, Raphael KG, Lavigne GJ, Janal MN, Sirois DA, Wigren PE, et al. Polysomnographic investigation of sleep and respiratory parameters in women with temporomandibular pain disorders. *Journal of Clinical Sleep Medicine*. 2014;10(2):195–201.
5. Tinastepe N, Küçük BB, Oral K. Botulinum toxin for the treatment of bruxism. *Cranio - Journal of Craniomandibular Practice*. 2015 Oct 1;33(4):292–9.
6. de Holanda TA, Castagno CD, Barbon FJ, Costa YM, Goettems ML, Boscatto N. Sleep architecture and factors associated with sleep bruxism diagnosis scored by polysomnography recordings: A case-control study. *Arch Oral Biol*. 2020 Apr 1;112.
7. Shim YJ, Lee HJ, Park KJ, Kim HT, Hong IH, Kim ST. Botulinum toxin therapy for managing sleep bruxism: A randomized and placebo—controlled trial. *Toxins (Basel)*. 2020;12(3).
8. Lobbezoo F, Ahlberg J, Raphael KG, Wetselaar P, Glaros AG, Kato T, et al. International consensus on the assessment of bruxism: Report of a work in progress. Vol. 45, *Journal of Oral Rehabilitation*. Blackwell Publishing Ltd; 2018. p. 837–44.
9. Castorflorio T, Deregibus A, Bargellini A, Debernardi C, Manfredini D. Detection of sleep bruxism: Comparison between an electromyographic and electrocardiographic portable holter and polysomnography. *J Oral Rehabil*. 2014 Mar;41(3):163–9.
10. Hasegawa Y, Lavigne G, Rompré P, Kato T, Urade M, Huynh N. Is there a first night effect on sleep bruxism? A sleep laboratory study. *Journal of Clinical Sleep Medicine*. 2013;9(11):1139–45.
11. Giovanni A, Giorgia A. The neurophysiological basis of bruxism. Vol. 7, *Heliyon*. Elsevier Ltd; 2021.
12. Chen Y, Tsai CH, Bae TH, Huang CY, Chen C, Kang YN, et al. Effectiveness of Botulinum Toxin Injection on Bruxism: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Vol. 47, *Aesthetic Plastic Surgery*. Springer; 2023. p. 775–90.
13. de Siqueira JTT, Camparis CM, de Siqueira SRDT, Teixeira MJ, Bittencourt L, Tufik S. Effects of localized versus widespread TMD pain on sleep parameters in patients with bruxism: A single-night polysomnographic study. *Arch Oral Biol*. 2017 Apr 1;76:36–41.

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The possibilities of a non-contrast enhanced abbreviated MRI protocol for breast cancer detection

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Purpose: The role of the contrast-enhanced abbreviated MRI protocol for breast cancer detection is recognized by all radiologists. However, the establishment of a non-contrast enhanced abbreviated MRI protocol (NCEA-MRI) will save radiologist time, reduce both patients' stress, downside gadolinium-related side effects and may decrease the amount of invasive procedures. **Methods and materials:** This retrospective study includes 64 patients for the period January, 2022 – December, 2022, who had breast-related symptoms and underwent MRI. Their scans were reanalyzed by excluding the contrast-enhanced sequences. Distinguishing benign from malignant solid lesions relied only on diffusion-weighted images (DWI) and macroscopic signs such as shape, margins and architectural distortion from T2-weighted images (T2WI) and T1-weighted images (T1WI). **Results:** The total of solid lesions in the 64 MRIs were 96, after histopathological verification 80 (83.3%) of the lesions were benign and 16 (16.7%) were malignant. From both groups 9 (9.4%) lesions were suspicious on the NCEA-MRI and further examination was needed. After the biopsy 3 (33.4%) of them turned out to be fibroadenomas and 6 (66.6%) – cancers. **Conclusion:** We are optimistic that NCEA-MRI protocol will be able to provide reliable results and thus in the future it can be implemented in the screening and diagnostic strategy for breast cancer. Limitations A small number of patients was included, which can interfere the outcome of our study and a larger clinical trial is needed for further validation of the results

Keywords: MRI, breast, cancer

Purpose

According to the European Cancer Information System, breast cancer is the most commonly diagnosed malignancy in the European Union in 2022, with a rate of 13.8%. It's also the third most common cause of death after lung cancer and colorectal cancer (1). Breast investigation today includes several imaging modalities – mammography, sonography and magnetic resonance imaging (MRI).

MRI is a well-established imaging method for evaluation of the breast. It outperforms the other techniques in many ways and its role has been proven over the years. Indications for breast MRI include detection of breast cancer, evaluation of breast implants, search for occult cancer or unknown primary malignancy, assessment before neoadjuvant therapy or after surgery (2). MRI is recommended for patients with dense breasts as there is no overlap of structures compared

to mammography. In addition, it provides not only morphological but also functional information about a lesion.

The dynamic contrast enhanced-MRI (DCE-MRI) protocol consists of non-contrast and post-contrast sequences. In a recent paper, Schoub concludes that the sensitivity of fMRI is 98–100%, which means that it can be used to rule out malignancy with a negative predictive value of 100% (3). Recently, however, global trends have been moving towards more resource-efficient methods. One of the pitfalls of the full protocol is the need for intravenous gadolinium-based contrast media, which have known side effects. This also leads to longer image acquisition times with higher energy consumption, more reading time of sequences by the radiologist, more time spent by the patient in the machine and higher costs of the examination (4).

Many retrospective studies have shown

that gadolinium-based contrast media (GBCM) remain in the brain for a long time, particularly in the dentate nucleus and globus pallidus. There may also be an association between GBCM administration and nephrogenic systemic fibrosis in patients with renal impairment (5, 6).

In the search for safer, time- and cost-saving modalities, the question arose as to whether non-contrast MRI could be as reliable as contrast-enhanced MRI. The non-contrast enhanced abbreviated MRI (NCEA-MRI) protocol will save radiologist time, reduce patient stress and gadolinium-related side effects, and may reduce the number of unnecessary biopsies.

Purpose of this research is to share our results of NCEA-MRI protocol, based on macroscopic appearance and diffusion-weighted images (DWI) in the detection and differentiation of benign and malignant breast lesions.

Methods and materials

This retrospective study includes 64 patients, who underwent breast MRI examinations between January 2022 and December 2022. The inclusion criteria were age above 18, the presence of breast-related clinical symptoms, no previous history of cancer, no biopsy or treatment before the MRI scan, and available histologic results from the lesions.

All MR examinations were conducted using a 1.5 T MRI scanner (Magnetom Amira, Siemens Healthcare, Erlangen, Germany) with an 18-channel breast coil, in prone position of the patient. The sequences included non-fat-suppressed T1WI, fat-suppressed TIRM (turbo inversion recovery magnitude), DWI and fat-suppressed dynamic contrast-enhanced T1WI. The DWI was performed at 3 different b values – $b = 50, 500, 800 \text{ mm}^2/\text{s}$. The scans were reanalyzed by a

radiology resident excluding the contrast-enhanced images. Lesion differentiation and BIRADS score were determined based on the presence of diffusion restriction and macroscopic features on T1WI and TIRM.

Results

The study included 64 patients aged 46.5 ± 9 years (21–69 years), who had undergone MRI scans. A total of 96 solid lesions were identified, all of which were either core-needle biopsied or surgically removed.

After histological evaluation 80 (83.3%) of the lesions were found to be benign, while 16 (16.7%) were malignant.

Of the benign lesions – 74 lesions showed no diffusion restriction. However, in 6 of these cases, the DWI and macroscopic features were insufficient to rule out malignancy. Out of the suspicious lesions two were found to be malignant.

In the group of malignant lesions 13 of them had typical malignant features on contrast-free sequences, including an irregular shape, speculations and diffusion restriction. Three lesions, which were not initially classified as malignant, actually turned out to be cancerous.

In total, nine (9.4%) lesions were suspicious on the NCEA-MRI. After the biopsy 3 (3.1%) of them were found to be fibroadenomas and 6 (6.3%) – cancers.

Discussion

In T1WI and TIRM, the lesions are analyzed based on their macroscopic appearance. Benign features include round or oval shape, circumscribed margins, homogeneity, no associated features. Suspicious for malignancy findings are irregular shape, non-circumscribed or spiculate margins, heterogeneity, architectural distortion, skin or nipple retraction, ductal changes, surrounding edema.

DWI is a fast MRI technique, that does not require intravenous contrast media, making it a cheaper tool. It is based on the movement of water within a certain amount of tissue. In malignant lesions the higher cellularity leads to restricted movement of the molecules. Using different b values the apparent diffusion coefficient (ADC) can be calculated. The ADC is a quantitative measure of

the restricted diffusion in a single voxel and is measured in mm^2/s . (1019, 1041) In breast cancer, there is a high signal on DWI and a low ADC value (7, 8) Figure 1 and 2.

In their study, Muzhen He et al., stated that ADC alone is a useful, cost-effective tool for differentiating benign and malignant lesions with a sensitivity 91.45% and specificity of 82.54% (9).

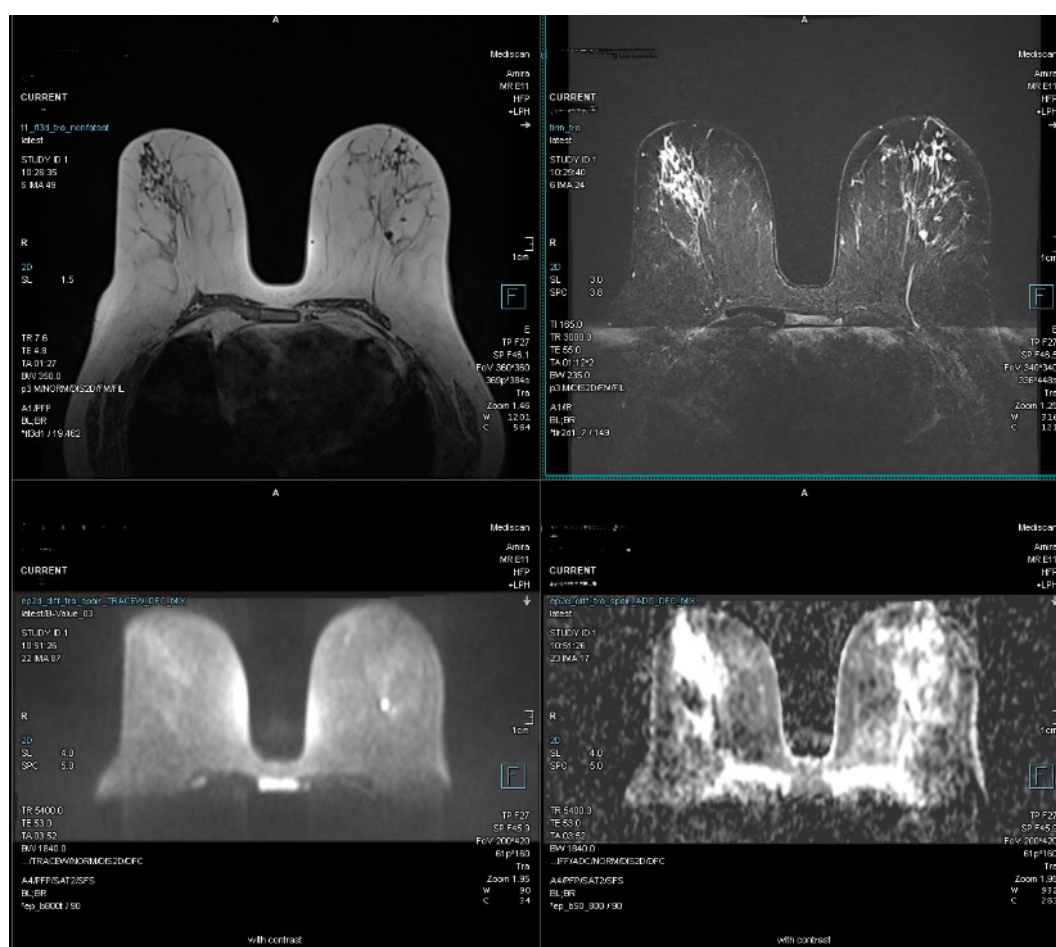


Figure 1. 69-year-old woman with a family history of breast cancer. On T1WI hypointense oval lesion with circumscribed margins, well-defined, homogenous, no associated features, no restricted diffusion, high ADC value. Histology – fibroadenoma.

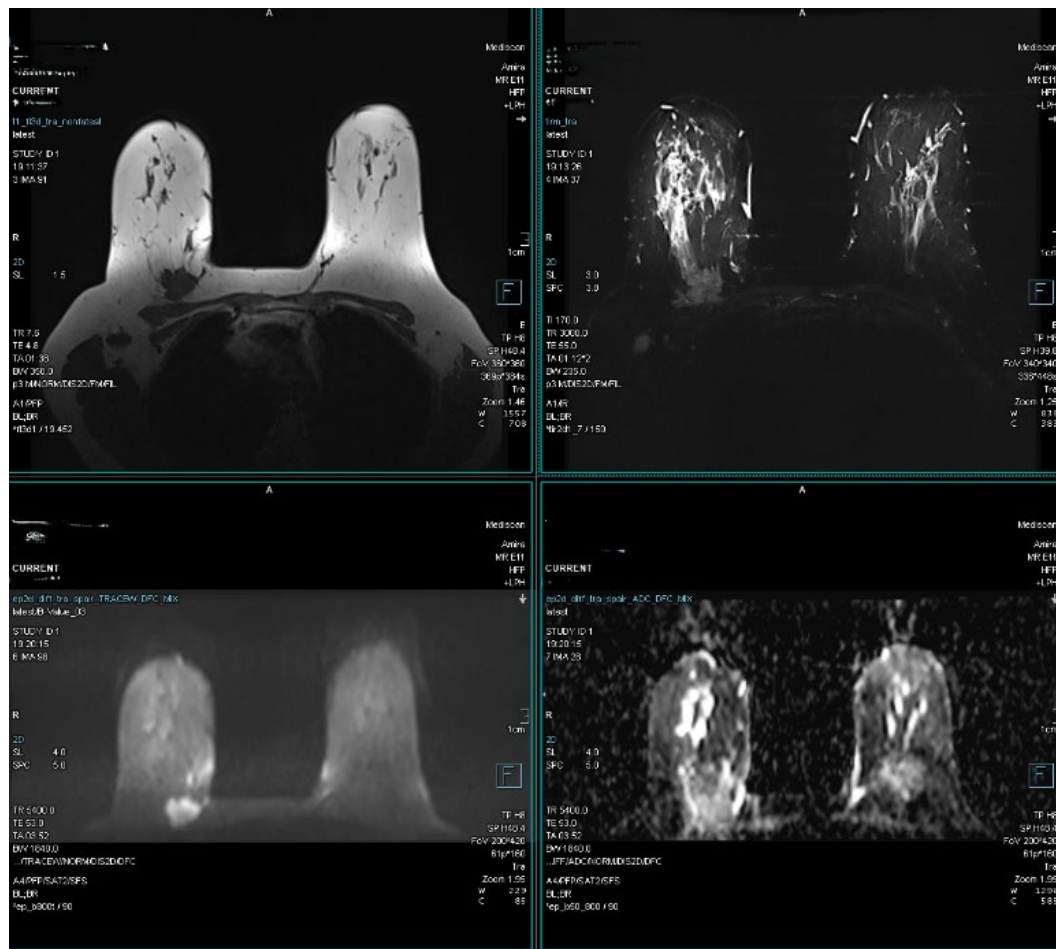


Figure 2. Breast MRI in a 47-year-old woman after BIRADS 5 lesion was found on mammography. On T1WI hypointense lesion with irregular shape, spiculated margins, a large lymph node in right axilla, restricted diffusion, low ADC value. Histology – invasive ductal carcinoma.

According to our retrospective analysis of 64 breast MRI examinations, the vast majority of lesions 87 (90.6%), could be accurately differentiated as either benign or malignant, based on their macroscopic features and the presence of restricted diffusion. A similar specificity of DWI was reported by Rotili et al. in their paper, 90% (6). However, in 9 lesions, the non-contrast enhanced protocol was not able to correlate with the final histology of the pathology. The reason for presence of false negative results has been widely discussed in the literature and several reasons have been suggested. Firstly, the absence of restricted diffusion may be related to histologic types of the malignancy. Many studies have highlighted that ductal carcinoma in situ (DCIS) has higher ADC

and can be easily missed. However, DCIS is a challenge not only for NCEA-MRI but also for DCE-MRI as it may not enhance on the post contrast series. The mucinous cancer contains more water and may also present with higher ADC values (2, 6). Secondly, there is probably a correlation between the ability to detect restricted diffusion and the size of the pathology. The reason for this pitfall is probably the spatial resolution of the images, which is lower on DWI. Thus, smaller malignant lesions may not be detectable on DWI sequence alone (10). Therefore, larger studies with subdivision of the lesions according to their size and histological type should be conducted.

Rates of false positive cases are more common with DCE-MRI, when benign

lesions are enhanced on post contrast sequences (2). This leads to a higher number of unnecessary biopsies of complicated cysts or fibroadenomas. According to a meta-analysis by Xin Chen et al., DWI has lower sensitivity, but higher specificity compared to contrast-enhanced MRI. Consequently, DWI should be a better tool for differentiating benign from malignant breast lesions and could reduce the number of biopsies after MRI examination (6, 7).

Because of the limitations of contrast-enhanced MRI, such as gadolinium-side effects, higher cost, longer reading time and more benign biopsies, the NCEA-MRI should be considered as an alternative for the evaluation of breast lesions. It is hoped that this resource – efficient protocol will be used as a supplemental tool to other modalities such as mammography and ultrasound for breast cancer screening in high-risk patients and women with dense breasts.

Conclusion

Given the limitations of DCE-MRI, NCEA-MRI may be used as a reliable, resource-saving tool for differentiating benign from malignant breast lesions. In the future, randomized control trials are needed to support this statement and to integrate NCEA-MRI examination into clinical practice.

Limitations

A small number of patients were included and the lesions were not grouped according to their size or histological type.

Bibliography

1. ECIS – European Cancer Information System <https://ecis.jrc.ec.europa.eu>
2. Scaranelo AM. What's Hot in Breast MRI. *Can Assoc Radiol J.* 2022 Feb;73(1):125-140. doi: 10.1177/08465371211030944. Epub 2021 Aug 12. Erratum in: *Can Assoc Radiol J.* 2022

May;73(2):433. PMID: 34384041.

3. Schoub PK. Understanding indications and defining guidelines for breast magnetic resonance imaging. *SA J Radiol.* 2018 Oct 30;22(2):1353. doi: 10.4102/sajr.v22i2.1353. PMID: 31754513; PMCID: PMC6837823.
4. Houser M, Barreto D, Mehta A, Brem RF. Current and Future Directions of Breast MRI. *J Clin Med.* 2021 Nov 30;10(23):5668. doi: 10.3390/jcm10235668. PMID: 34884370; PMCID: PMC8658585.
5. Davies J, Siebenhandl-Wolff P, Tranquart F, Jones P, Evans P. Gadolinium: pharmacokinetics and toxicity in humans and laboratory animals following contrast agent administration. *Arch Toxicol.* 2022 Feb;96(2):403-429. doi: 10.1007/s00204-021-03189-8. Epub 2022 Jan 8. Erratum in: *Arch Toxicol.* 2022 Feb 28;: PMID: 34997254; PMCID: PMC8837552.
6. Rotili A, Pesapane F, Signorelli G, Penco S, Nicotia L, Bozzini A, Meneghetti L, Zanzottera C, Mannucci S, Bonanni B, Cassano E. An Unenhanced Breast MRI Protocol Based on Diffusion-Weighted Imaging: A Retrospective Single-Center Study on High-Risk Population for Breast Cancer. *Diagnostics (Basel).* 2023 Jun 7;13(12):1996. doi: 10.3390/diagnostics13121996. PMID: 37370892; PMCID: PMC10297064.
7. Chen, X., Li, Wl., Zhang, Yl. *et al.* Meta-analysis of quantitative diffusion-weighted MR imaging in the differential diagnosis of breast lesions. *BMC Cancer* **10**, 693 (2010). <https://doi.org/10.1186/1471-2407-10-693>
8. Tsvetkova, S.; Doykova, K.; Vasilksa, A.; Sapunarova, K.; Doykov, D.; Andonov, V.; Uchikov, P. Differentiation of Benign and Malignant Breast Lesions Using ADC Values and ADC Ratio in Breast MRI. *Diagnostics* **2022**, *12*, 332. <https://doi.org/10.3390/diagnostics12020332>
9. He M, Ruan H, Ma M, Zhang Z. Application of Diffusion Weighted Imaging Techniques for Differentiating Benign and Malignant Breast Lesions. *Front Oncol.* 2021 Jun 21;11:694634. doi: 10.3389/fonc.2021.694634. PMID: 34235084; PMCID: PMC8255916.
10. Amornsiripanitch N, Bickelhaupt S, Shin HJ, Dang M, Rahbar H, Pinker K, Partridge SC. Diffusion-weighted MRI for Unenhanced Breast Cancer Screening. *Radiology.* 2019 Dec;293(3):504-520. doi: 10.1148/radiol.2019182789. Epub 2019 Oct 8. PMID: 31592734; PMCID: PMC6884069.

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Trichoscopy in the diagnosis of alopecia areata

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Introduction: Alopecia areata (AA) is one of the most common hair loss disorders. The prevalence of the disease is 0.1–0.2% of the general population. AA is a T-cell mediated autoimmune condition characterized by non-scarring hair loss that may affect only parts of the scalp, the entire scalp or parts of the body. AA may manifest as a single episode or may have a relapsing course. Dermoscopy of the hair and scalp is known as trichoscopy: a useful method for diagnosing and monitoring hair and scalp disorders, as well as monitoring the effect of the applied treatment. **Aim:** To make a review of the literature on the topic of trichoscopy in the diagnosis of alopecia areata. **Materials and methods:** PubMed publications were searched using the following keywords: “trichoscopy”, “alopecia areata”. A total of 143 publications published between 2008 and 2023 were reviewed. **Results and discussion:** The presence of exclamation mark hairs, black dots, triangular hairs, broken hairs, and pointed hairs in the alopecic patches indicates disease activity. One of the most common trichoscopic features of AA are the yellow dots – empty follicular openings filled with keratin and sebum. They predominate in long-standing, inactive disease. Short vellus hairs are also common. Hair regrowth phase includes: upright regrowing hairs and circle hairs. Diagnosis should be based on the coexistence of several trichoscopic findings rather than the presence of a single sign. **Conclusion:** Trichoscopy is a useful method for diagnosing and monitoring of AA, as well as for follow-up after treatment.

Keywords: alopecia areata, diagnosis, trichoscopy, dermoscopy, hair loss

Introduction

Alopecia areata (AA) is one of the most common non-scarring hair loss disorders that has a prevalence of 0.1–0.2% in the general population. AA is an autoimmune T-cell mediated condition and may manifest as a single episode or may have a relapsing course. It may affect only parts of the scalp, the entire scalp or parts of the body. The diagnosis of AA is based on clinical examination and trichoscopy [17, 18, 19].

Aim

To make a review of the literature on the topic of trichoscopy in the diagnosis of alopecia areata.

Materials and methods

PubMed publications were searched using the following keywords: 'trichoscopy', 'alopecia areata'.

Results

A total of 143 publications published between 2008 and 2023 were reviewed.

Discussion

Dermoscopic examination of the hair and scalp is known as trichoscopy. The term was

introduced by Lidia Rudnicka and Malgorzata Olszewska in 2006. Trichoscopy is a non-invasive method, a variation of dermoscopy, in which the scalp and hair are examined with a standard dermatoscope (x10 magnification) or a digital videodermatoscope (x20–1000 magnification). It is a useful method in diagnosing and monitoring hair and scalp disorders, and in monitoring the effect of the treatment [1–7].

Trichoscopy helps in the diagnosis of AA. The most well-known trichoscopic feature of AA is the exclamation mark hairs (Fig. 2). These are short hairs with a thick and dark distal end and a narrowed light proximal end. The hair shaft appears to be stuck into the follicular opening. These trichoscopic markers are result of the dystrophic mechanism of the hair condition. The presence of exclamation mark hairs at the borders of the alopecic areas indicates the activity of the disease [1–3, 8–10].

Other trichoscopic features of an active AA are:

- black dots: pigmented hairs interrupted or destroyed at the scalp level. They correspond to remnants of hair shafts which, due to a disturbed anagen phase, are broken

even before they emerge above the skin surface (Fig. 1);

- triangular hairs: they have a distal pointed end and a proximal portion hidden by a whitish-gray veil. Presumably, like black dots, they are remnants of broken hairs, exclamation mark hairs, or tapered hairs;

- broken hairs: they are observed in 46% of patients. These are short hairs with normal hair shafts, but with irregular, choppy distal ends. They result from an irregular transverse breakage of the terminal hair shaft due to an inflammatory process or from rapid outgrowth of incompletely broken hairs that have previously formed black dots;

- tapered hairs: long exclamation mark hairs with the proximal end tapered and the distal end unable to be caught by the lens of the dermatoscope (Fig. 4);

- Pohl-Pincus constrictions: areas of reduced hair thickness of the hair shaft. These constrictions occur most commonly when the metabolic and mitotic activity of hair follicle is rapidly and repeatedly suppressed [3, 9, 11–14].

Another common trichoscopic sign of AA is the yellow dots (Fig. 3). This finding predominates in long-standing, inactive disease. The yellow dots represent empty follicular openings filled with keratin and sebum. In AA, they appear in groups reflecting the number of hairs in the follicular units. This marker can also be present in the acute form of AA. Short vellus hairs, unpigmented hair shafts less than 10 mm in length and less than 0.03 mm in thickness, are also common [1, 9] (Fig. 3). Short vellus hairs are less than 10 mm long. They represent hairs that are unable to continue their anagen growth. Normal-appearing hairs that can easily break when bent or pressed have also been described in AA. This phenomenon is called “coudability”, meaning “elbow effect” [16]. These “coudability hairs” are also associated with AA activity [12].

Hair recovery can be monitored by trichoscopy with the following signs: upright regrowing hairs (anagen hairs, which are pointed and grow in upright position), pigtail hairs and coiled hairs (uniformly curved in the distal part). Pigtail hairs are short, growing, properly curled and with pointed ends. This is a rare finding associated with hair outgrowth in patients with acute hair loss. These hairs differ from pigtail hairs by their irregular structure and choppy distal end [3, 9, 14, 15].

Other interesting trichoscopic signs that have been seen less frequent are:

- honeycomb pattern: it consists of grouped and homogeneously colored brown oval spots. It is observed in chronic sun exposed areas and in dark-skinned individuals.

- dirty dots: dirty dots are non-microbial particles from the environment and are easily removed after shampooing. They may be brown, black, red, yellow or blue, and may look like fibers.

- white dots: two types of white dots are described, large and small. Large white dots occurring in chronic AA correspond to areas of follicular fibrosis. Smaller white dots correspond to the openings of eccrine sweat glands.

- tulip hairs: short tulip leaf-shaped hairs with hyperpigmentation at the distal end [3, 4, 9] (Fig. 2).

Trichoscopy is a useful tool in assessing not only disease activity, but also severity and duration. More precisely, severe active AA is characterized by the presence of black dots, yellow dots, reduced amount of vellus hairs, honeycomb pattern, clusters of white dots. In the mild active AA there are only exclamation mark hairs. Acute AA has the presence of exclamation mark hairs, black dots and vellus hairs, while chronic AA features yellow dots, smooth and fine hair on the scalp and very weak growth of pigmented hairs in homogeneous distribution [3].

There are different types of the condition, ranging from small single alopecic patches to absence of all hairs on the body. It is described that AA incognita features extensive amount of pigtail hair (57.9%), newly growing hairs, black dots, yellow dots distributed diffusely, mainly affecting the occipital and parietal region, and proportionally to the severity of the hair loss. In AA diffusa the inflammatory damage to the follicles is much

greater than in AA incognita. There is greater presence of dystrophic hairs, black dots (36%), and yellow dots in the parietal and anterior-temporal areas of the scalp. In the ophiasis AA, the trichoscopy shows mainly black dots and there are no yellow dots. Positive prognostic markers for alopecia totalis and universalis include pigmented vellus hairs and upright regrowing hairs [3].



Figure 1. Black dots (Dino-Lite Edge AM7915MZT(R7) x70, images Lidiya Todorova)

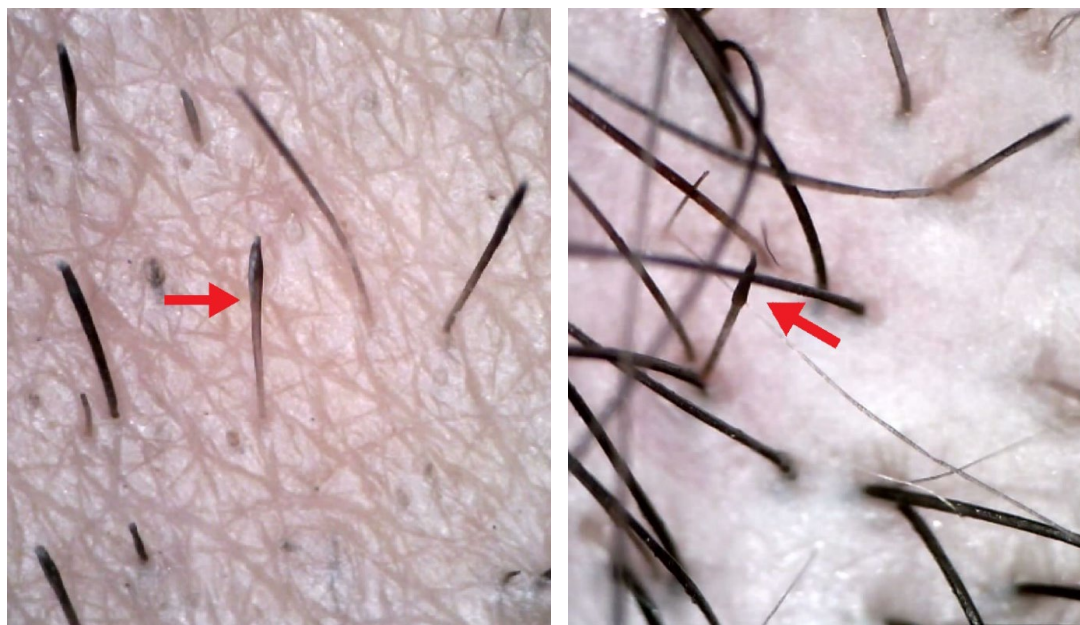


Figure 2. Exclamation mark hairs (left) and tulip hairs (right) (Dino-Lite Edge AM7915MZT(R7) x70, images Lidiya Todorova)

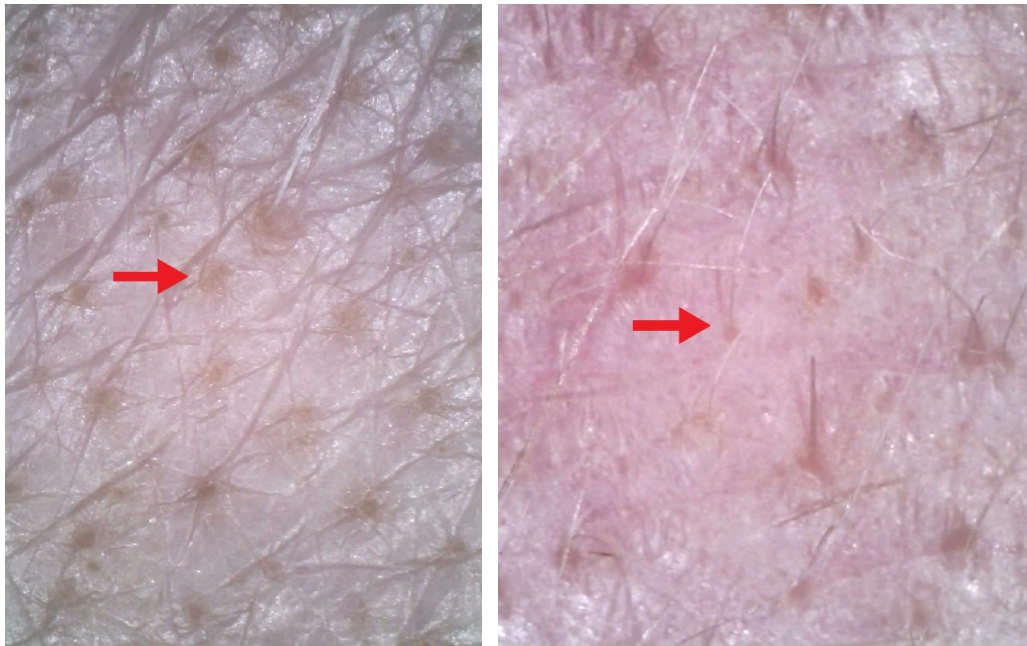


Figure 3. Yellow dots (left). Vellus hairs (right) (Dino-Lite Edge AM7915MZT(R7) x70, images Lidiya Todorova)



Figure 4. Tapered hairs (Dino-Lite Edge AM7915MZT(R7) x70, image Lidiya Todorova)

Conclusion

Trichoscopy is a useful method to diagnose and monitor AA. The diagnosis of AA should be based on clinical examination and trichoscopy. The trichoscopic evaluation should take into account the simultaneous existence of several trichoscopic findings rather than the presence of a single sign.

References

1. Dobrev H. Application of trichoscopy in clinical practice. Health.bg 2013; 9: 34-37. [ISSN 1314-2569] [In Bulgarian]
2. Dobrev H. Application of dermoscopy (trichoscopy) in mycosis of the scalp. GP News. 2016; 10: 24-25 [In Bulgarian]
3. Gómez-Quispe H et al, Trichoscopy in Alopecia Areata. Actas Dermosifiliogr. 2023; 114(1): 25-32.
4. Miteva M, Tosti A. Hair and scalp dermatoscopy. J Am Acad Dermatol. 2012; 67(5): 1040–1048.

5. Olszewska M et al, Trichoscopy. *Arch Dermatol*. 2008; 144(8).
6. Rudnicka L et al, Presence and future of dermoscopy [meeting report]. *Expert Rev Dermatol*. 2006; 1(6):769-772.
7. Wałkiel-Burnat A et al, Trichoscopy of alopecia areata in children. A retrospective comparative analysis of 50 children and 50 adults. *Pediatr Dermatol*. 2019; 36(5): 640-645.
8. Lacarrubba F et al, Videodermoscopy enhances diagnostic capability in some forms of hair loss. *Am J Clin Dermatol*. 2004; 5(3): 205-8.
9. Rudnicka L et al, Atlas of Trichoscopy: Dermoscopy in Hair and Scalp Disease. Springer, London, 2012.
10. Rudnicka L et al, Hair Shafts in Trichoscopy. *Dermatologic Clinics*. 2013; 31(4): 695–708.
11. Guttikonda AS et al, Evaluation of Clinical Significance of Dermoscopy in Alopecia Areata. *Indian J Dermatol*. 2016; 61(6): 628-633.
12. Inui S et al, Clinical significance of dermoscopy in alopecia areata: analysis of 300 cases. *Int J Dermatol*. 2008; 47(7): 688–693
13. Rudnicka L et al, Trichoscopy update 2011. *J Dermatol Case Rep*. 2011; 5(4): 828-828.
14. Wałkiel-Burnat A et al, Alopecia areata predictive score: A new trichoscopy-based tool to predict treatment outcome in patients with patchy alopecia areata. *J Cosmet Dermatol*. 2020; 19(3): 746-751.
15. Wałkiel A et al, Trichoscopy of alopecia areata: An update. *J Dermatol*. 2018; 45(6), 692–700.
16. Shuster S. “Coudability”: a new physical sign of alopecia areata. *Br J Dermatol*. 1984; 111(5): 629–629.
17. Meah N et al, The Alopecia Areata Consensus of Experts (ACE) study: Results of an international expert opinion on treatments for alopecia areata. *J Am Acad Dermatol*. 2020; 83(1):123-130.
18. Rakowska A et al. Alopecia areata. Diagnostic and therapeutic recommendations of the Polish Dermatological Society. Part 1. Diagnosis and severity assessment. *Dermatology Review/Przegląd Dermatologiczny*. 2023; 110(2): 89-100.
19. Rakowska A et al. Alopecia areata. Diagnostic and therapeutic recommendations of the Polish Society of Dermatology. Part 2: Treatment. *Dermatology Review/Przegląd Dermatologiczny*. 2023; 110(2): 101-120.
20. Wałkiel-Burnat A et al, Trichoscopy of alopecia areata in children. A retrospective comparative analysis of 50 children and 50 adults. *Pediatr Dermatol*. 2019; 36(5): 640-645.

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Lactate as a modern tool of exercise physiology – a review of the literature

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Introduction: Lactate is a key molecule in exercise metabolism. In the last two decades there has been a revolution in the understanding of its role – from a byproduct of hypoxic muscles to a major energy resource and a signaling molecule – ‘lactormone’. **Aim:** The aim of this review is to compile all the up-to-date information that is available on the general metabolism of lactate, but also the specific use that exercise physiologists, coaches and athletes can get out of lactate measurements, lactate-based training zones and periodization of training sessions. **Materials and methods:** Three databases were searched for this review – PUBMED, Google Scholar and Science Direct. **Results and discussion:** Since the revolution in the understanding of lactate’s role in normal metabolism and disease in the late 20th century, more evidence has been assembled regarding the specific part it plays in exercise. From the vast body of knowledge was developed the concept of training intensity distribution into zones according to the level of blood lactate. The end goal of training to a specific lactate level or ‘threshold’ is the increased utilization of lactate as an energy source by active muscles and ultimately better performance, which has been backed up by the achievements of numerous athletes, training according to this concept. **Conclusion:** Lactate is established as a valuable training tool, but nevertheless it is part of an evolving scientific field, which changes rapidly and an updated look at the science behind lactate-based training can be beneficial to athletes overall.

Keywords: *lactate, exercise, threshold, OBLA, LT*

Introduction

Lactate was first discovered as a molecule in 1780 by Carl Wilhelm Scheele in milk, who named it ‘Mjölksyra’ which was later translated to lactic acid. In the next two centuries as a result of numerous studies, namely by Pasteur, Warburg, Cori and Claude Bernard to name a few, a correlative relationship was established between the production and accumulation of lactate and the amount of oxygen that is available to cells for their metabolic processes. This ‘classical view’ of lactate metabolism led to inception of the dogma that its production is solely related to hypoxia, which was later extrapolated to exercise physiology and muscle metabolism during exercise, despite the fact that extensive research conceived results that contradicted this, but never challenged it (1). Still that view persisted through the majority of the twentieth century and it made its way firmly into exercise physiology, where the term ‘anaerobic threshold’ was coined in the 1960’s by Wasserman et al. They defined it as the metabolic

point during exercise where blood lactate concentration starts to increase and blood bicarbonate concentration starts to decrease (2). It wasn’t until the early 1980’s that this concept was put to the test by Brooks et al., who demonstrated that lactate not only is not a metabolic waste and marker of anaerobiosis, but it was produced under fully aerobic conditions and had the potential of a universal cellular fuel (3). While detailed lactate metabolism was still not clear, other researchers in the late 70’s were starting to put their findings into practice. Kindermann et al. were one of the pioneers of using lactate levels measured during exercise as a marker of intensity, which could be quantified and used to prescribe training at specific exercise intensities for enhancing performance capacity (4). Although, using lactate as an effective training tool has been around for more than four decades, it has only gained traction recently, thanks to the achievements of the Ingebrigtsen brothers in running, Kristian Blummenfelt in triathlon, Tadej Pogačar in cycling and many other elite athletes who have

demonstrated that lactate-based training can bring tremendous performance results (5–7).

Aim

The principal target of this review is to compile and describe in understandable detail the latest discoveries in lactate metabolism, to compare them to the original thought of what lactate was considered as and to highlight from that body of information the most useful practices that are applied and that can be added to the use of lactate metabolism in exercise physiology and training. Understandably this is aimed at athletes, coaches and exercise scientists who wish to understand some of the science behind lactate-based training, but also for medical practitioners who wish to update their knowledge of lactate.

Methods and results

Biomedical databases were searched for the purpose of this review – PubMed, Scopus, Web of Science and Google Scholar. The search terms ‘lactate’, ‘lactic acid’, ‘threshold’, ‘intensity’, ‘endurance’,

‘aerobic’, ‘anaerobic’, ‘metabolism’, ‘exercise’ were used in various combinations. The search yielded 49 articles of different types such as case reports, literature reviews, original research articles and systematic reviews.

Discussion

Origin and nature of lactate and its ‘classical’ metabolism

Lactate, also known as lactic acid, is an organic molecule that is produced in the process of glucose breakdown, named glycolysis. This catabolic process is widespread in all cellular types, from prokaryotes to complex organisms. In human cells glycolysis terminates in one main product – pyruvate, which can be further metabolised in mitochondria and the Krebs cycle to yield ATP molecules, or can be transformed into lactate by lactate dehydrogenase (LDH) in the cytosol (8). To this point in the metabolism of lactate the consensus of its origin is unanimous, however from here onwards there has been a major paradigm shift in the direct causes for production and fate of lactate.

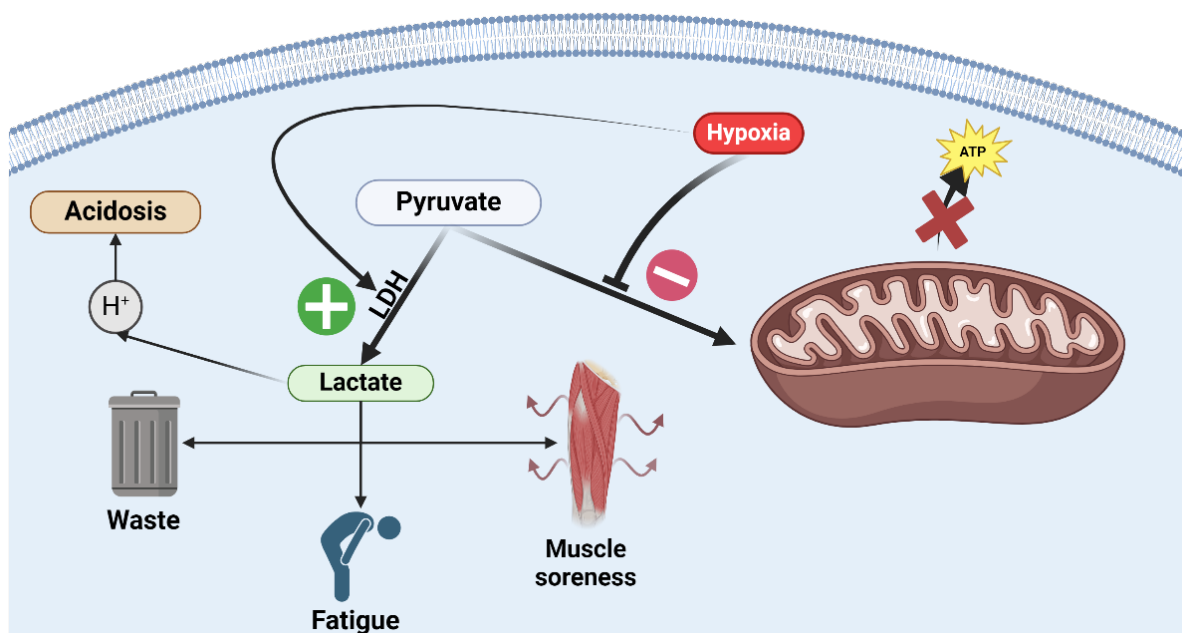


Figure 1. Traditional views on lactate metabolism

The ‘classical’ understanding of lactate production has always been that its levels increase dramatically when cells experience hypoxia and glycolysis terminates at the pyruvate step, thereby shifting the LDH reaction towards lactate. As early as 1807 Berzelius noted that the ‘muscles of hunted stags’ contained some form of acid. Later research in the 19th and 20th century cemented that view and showed that this acid was lactic, and in muscles it originated from glycogen, hence that it was a product of glycolysis. It was also found that muscles deprived of oxygen produced much more lactate and were much more acidic, which led to a simple intuitive conclusion that hypoxia should be the driving force behind increased lactate production. This view was reinforced in the research of prominent scientists like Nobel

laureates Meyerhof and Hill, hence why it took a long time to be challenged (1).

Another probable misconception of lactate has arisen regarding its biochemical role in cellular acidosis owing to exercise. Recently Robergs et al. have argued that in vivo it is impossible for lactic acid to be a product of glycolysis, since acids are molecules containing hydrogen atoms that they can donate and at the physiological pH of cells acids are produced in their deprotonated form, hence why Robergs et al. are adamant there is no such product of cellular metabolism known as lactic acid (9–11). Although it is not the objective of the current review to explain this debate in acid-base balance it is a matter worth exploring, especially since it relates to the origin of a prominent medical complication in the face of metabolic acidosis.

Newer perspectives of lactate metabolism

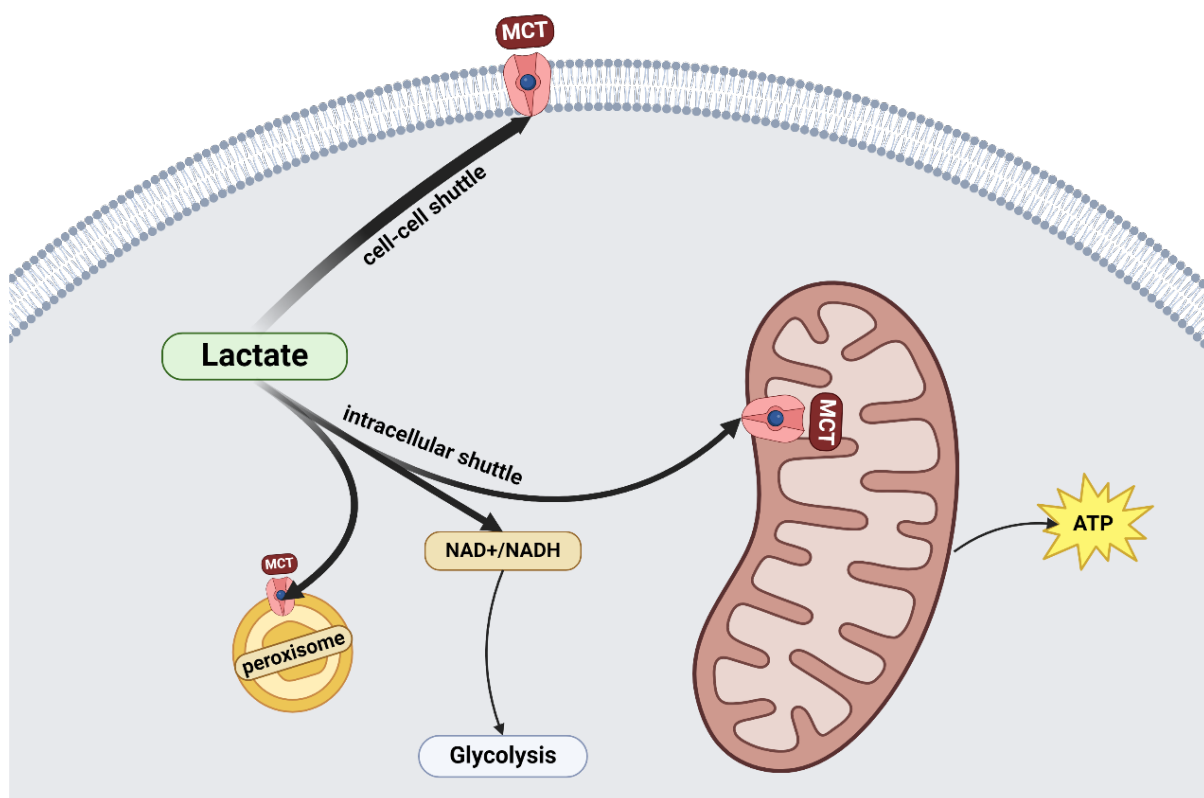


Figure 2. New hypotheses of lactate metabolism

Since the 1980's a heap of new research has thwarted the traditional thought of what lactate actually is. The proposal of the lactate shuttle theory by George Brooks has significantly shifted the paradigm of lactate understanding – from a waste product and fatigue-inducer to a universal cellular fuel and a key metabolic 'cog' (12). The gist of this hypothesis is that lactate is not produced in challenging conditions through only anaerobic glycolysis, but rather it is a major product during rest in normoxic conditions and even more so during exercise. This lactate is then 'shuttled' intracellularly but also to numerous organs and tissues which can directly oxidise it and use it for ATP production, as well as in the process of gluconeogenesis, all of that depending on the specific cellular needs. Apart from its strictly energetic role, lactate can also serve as a signalling molecule (13).

One of the basic tenets of the cell-cell lactate shuttle hypothesis is the discovery of the monocarboxylate transporter (MCT) system – the proteins which transport lactate between different cells and the blood, but also between different organelles in the same cell by facilitated diffusion (14). These MCTs are ubiquitously found in cell membranes and are further subdivided into more than 14 types, which transport not only lactate, but also pyruvate, short-chain fatty acids, and monocarboxylate drugs (15).

Entering in more detail, it can be said that lactate's role in cellular energetics is now well established. It is demonstrably a regular product of glycolysis, as shown by the ratio of lactate to pyruvate, which even in resting muscle is above 10. Moreover, extensive research has shown net lactate release from exercising muscles at tissue partial pressure of oxygen at around 40 mmHg (Torr), which is well above the critical mitochondrial pO_2 of 1–2 mmHg (Torr) – another piece of evidence against the notion that lactate

production is anaerobically dependent (16). Lactate kinetics have been investigated during rest and exercise. In the resting organism the rate of appearance (R_a) of lactate, i.e. its production, matches its rate of disposal (R_d), i.e. its clearance from the blood. When transitioning to exercise, some muscles and tissues will become net lactate producers, while others become net consumers – with that the levels of blood lactate increase as a reflection, but depending on the intensity of exercise they can reach a new steady state. On the other hand further increase in intensity of exercise will be reflected in dramatically increased lactate levels, when the R_a vastly overcomes the R_d (17). The proposed explanation lies in the crossover concept of fuel utilisation during exercise – from predominantly lipids in light intensity activities, switching steadily to carbohydrates in increasing intensities, aiming again to escape from the notion that dysoxia is the driver of lactate production and cementing the understanding that it is directly dependent on the rate of glycolysis (18). On the other hand, lactate disappearance by oxidative clearance is thought to be its main metabolic fate in tissues with low and moderate oxygen and ATP consumption during rest and exercise and it happens either in the cytosol (by LDH conversion to pyruvate) or directly in the mitochondria. Contemporary evidence is now rather pointing to mitochondrial oxidation being the most likely path, but it is still a topic of debate whether a mitochondrial LDH is capable of producing the observed levels of clearance (13, 19). Examples of the cell-cell shuttles include the exchange of lactate between working muscles and the heart, brain and liver, red and white oxidative muscle fibres, astrocytes and neurons, etc. The intracellular shuttles on the other hand include that of lactate exchange between the cytosol, mitochondria and peroxisomes (13).

Gluconeogenesis is the other main metabolic role that can be attributed to lactate, especially at rest, when more than 80% of lactate production is directed towards glucose resynthesis through the Cori cycle, having in mind that this is observed in the fed and postprandial state (the glucose paradox), when glycolysis levels are high enough (16). During a fasted state, the precursors of gluconeogenesis shift predominantly to other three-carbon compounds such as glycerol (20).

Last, but not least, recent studies have investigated the proposition that lactate is a major signalling molecule. It first stems from the fact that it exists in millimolar concentrations in the blood. It has been appreciated that lactate supposedly stimulates and binds a myriad of cytosol targets which regulate cellular metabolism and immunity – PGC-1 α , HCAR-1, SIRT-1, SIRT-3, etc. The downstream physiological effects of these interactions include mitochondrial biogenesis, lipid metabolism control, immune system control, etc (21). These pathways however can be maladaptive when activated in pathologically transformed cells, such as in cancer, where lactate metabolism is of gross importance to their survival and malignant behaviour like metastasis (22).

Lactate in exercise

It has already been established that lactate possesses a prominent role in energetics during exercise. As a product of glycolysis, its concentration rises steadily with the increasing rates of carbohydrate oxidation during physical activity and it can reach high values of approximately 20 mmol/l. Early research designated a correlation between the changes in blood lactate levels during exercise and the intensity of exercise or the amount of work that was being done (23). Although that relation was based on the assumption of a dysoxia-lactate dependence, it

correlated well with other physiological determinants of physical exertion – mainly gas exchange, and that assumption stuck in the literature. These conclusions on the role of lactate in muscle were based on the research of prominent physiologists like Meyerhof, Fletcher, Hopkins, Hill, Dill and many others and it is important to note that current understanding does not dismiss, but builds upon that knowledge (24). We now understand the basic metabolism of lactate and its kinetics during exercise and appreciate that glycolysis and increased carbohydrate oxidation is the driver of lactate accumulation in the blood, while hypoxia may play a secondary role. On the other hand, even during physical activity lactate can be cleared via shuttling and oxidation – a quality that improves with higher levels of aerobic capacity, thus it is often used as a marker of improved performance (25, 26). This adds to debunking the ‘myth’ of lactate’s role in fatigue, since it is cleared rapidly after cessation of exercise. Contemporary research identifies ATP depletion, oxygen insufficiency, impaired calcium kinetics, phosphate accumulation and neural mechanisms as main contributors to muscle fatigue, rather than lactate and its related acidosis (27).

The lactate threshold

As mentioned above in the early 20th century physiologists started using lactate as a measurement of intensity and plotting it against the work done during exercise thus constructing the concept of the blood lactate curve. The first one to notice a specific point on that curve which signified an increase in the lactate concentration, owing to an increase in exercise intensity was W. H. Owles, which more or less is the first instance a ‘lactate threshold’ was acknowledged, although the term wasn’t coined for another 35 years (24, 28). Scientists continued investigating

the response of lactate concentration in the blood to exercise, and the relation to intensity was further solidified by studies which showed the different lactate curves, matching graded intensity from light to severe (29). Still this remained a concept for the lab and it was not transferred as a training strategy in sport until much later.

The first ‘threshold’ of any kind that was introduced was the anaerobic threshold (AT), coined by Wasserman and McIlroy in 1964. They defined it as ‘the metabolic point where bicarbonate concentration in the blood decreases and lactate concentration increases’ (2). Although a valid assumption at the time, the researchers did not actually measure lactate levels in the blood – instead they measured the rate of gas exchange, the theoretical basis being that increased VCO_2 and respiratory exchange ratio (RER) above 1 signify the onset of anaerobic metabolism,

which at the time was directly thought to be coupled with a blood lactate increase. Later the V-slope method for calculating the AT was proposed by Wasserman and Beaver – the discrepancy between the rate of carbon dioxide production and oxygen consumption, which they thought would be an accurate non-invasive surrogate for determining the accumulation of lactate. What these researchers pioneered was the basis of the lactate threshold, but since it didn’t actually measure lactate, they introduced what is today been differentiated as the gas exchange threshold (GET) (24, 30). Last but not least an almost equivalent term in the face of ‘ventilatory thresholds’ (VT) was presented which determined the anaerobic threshold by the discrepancy between the rate of minute ventilation plotted against oxygen consumption or carbon dioxide production – the ventilatory equivalents (31, 32).

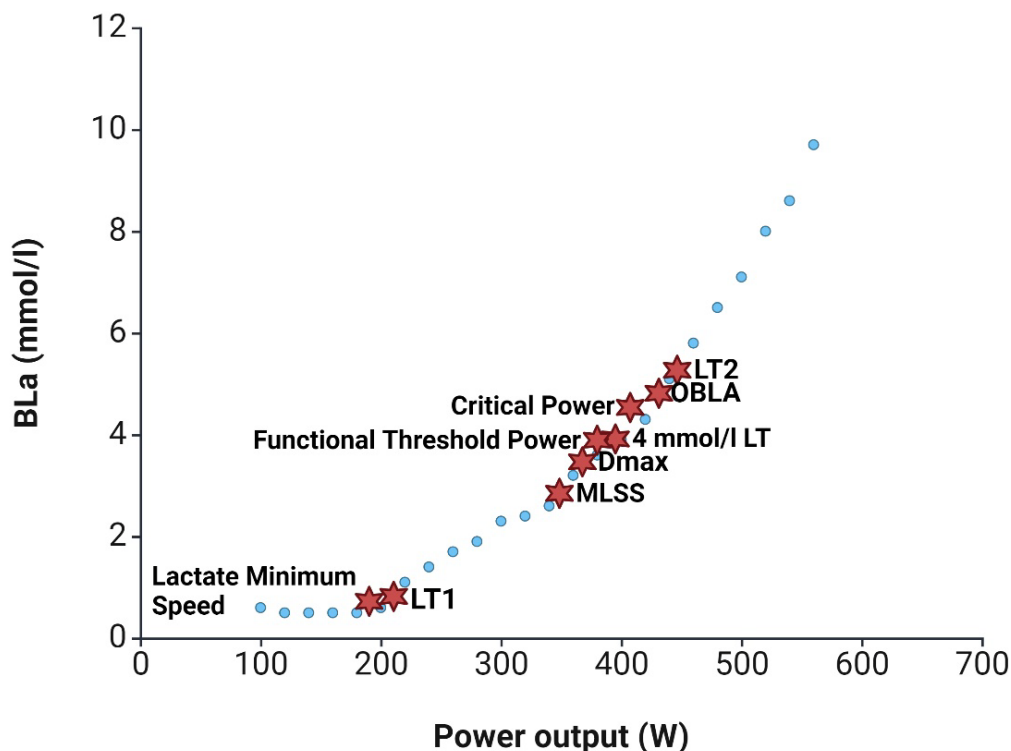


Figure 3. Concepts of lactate thresholds

The lactate threshold concept gained traction with the introduction of the enzymatic methods of measuring lactate from whole blood, which made it easier to assess lactate concentrations during laboratory exercise tests (33). The invention of the test strip methods for measurement by Shimojo et al. in the 1990's made it even more accessible and easy to implement and thus more popular between athletes (34, 35). This accelerated research on the lactate threshold, using direct blood lactate measurements, but in time it produced a glaring problem – the lack of standardisation and consensus on the terminology led to the inception of various threshold concepts, which aimed to describe the same physiological event but still differed enough so as not to be considered the same. As reviewed by Faude et al., up to 25 different LT concepts have been identified in the literature (33). Generally, in the literature are agreed upon and identified two main thresholds that signify two physiological events – the first LT which marks the increase of blood lactate above resting levels and the second LT which marks the non-linear exponential increase in lactate concentration, which is thought of as the point where muscle metabolic acidosis accelerates. However, the debate is still revolving around the nature and exact meaning of the lactate thresholds more so for the second LT, than the first. Some concepts, however, stand out from the others as more popular or more accurate for determination of the second LT – the maximal lactate steady state (MLSS), onset of blood lactate accumulation (OBLA), the 4 mmol/l LT, the Dmax and modified Dmax methods (36). MLSS is defined as the highest exercise intensity at which the arterial lactate concentration remains stable, albeit elevated from resting levels. The term was coined by Heck et al. who developed a protocol for identifying this metric, which

consists of several exercise bouts of ~30 minutes. Although accurate in measuring the aerobic-anaerobic transition around LT2, this method is not used often in everyday practice, as it's time and energy consuming (37). The OBLA is defined rather vaguely as the starting point of exponential increase in blood lactate concentration during graded exercise and it was again introduced to signify that metabolic shift (38). Mader et al. chose to show that shift with a specific value, hence why they developed the 4 mmol/l LT, which relies on the power at which a 4 mmol/l of lactate is reached in blood, to determine the second LT and that specifically had been an established long standing belief among exercise scientists of where LT2 is (39). The Dmax and modified Dmax are mathematical methods for calculating the second LT by identifying the point on the third order polynomial lactate curve, which lies at the longest perpendicular distance from a line, drawn between the highest and lowest measured lactate values on that curve. The method was proposed by Cheng et al. and modified by Bishop et al. (40, 41). All of those methods for determining the second threshold are valid, but the modified Dmax has been shown by Heuberger et al. to produce the least variance between successive calculations and also predict performance most accurately, when used to distribute intensity into zones, which are then used for exercise prescription (36).

Application of theory into practice

When the anaerobic threshold concept was introduced by Wasserman, the main idea behind it was to quantify the physical capacity of cardiac failure patients (2). The first ones to propose the threshold concept for enhancing athlete performance were Kindermann et al., who appreciated that different intensity of training elicited different

physiological responses and stimuli. They also acknowledged that the proposed ‘anaerobic’ threshold correlated with only slight increases in lactate up to 2 mmol/l and was actually a work rate that can be maintained for long periods of time, hence not anaerobic after all. This is why these authors proposed a new framework of two thresholds akin to the lactate thresholds discussed above – the aerobic and anaerobic threshold and also pioneered a line of thought of using that framework for prescribing exercise at different intensities, which could overall enhance performance (4). On that basis and other

research in the field in the 1970’s Skinner and McLellan further developed this idea into a three-phase model of intensity distribution in graded exercise, describing in detail changes in metabolism, muscle, cardiovascular and respiratory physiology (42). As discussed above, the nomenclature around the thresholds is quite unclear, owing to the fact that there is no standardisation, but the concept that Kindermann introduced and was further outlined and described by Skinner, has been tested in time and shown to be accurate enough for practical use and exercise prescription (43).

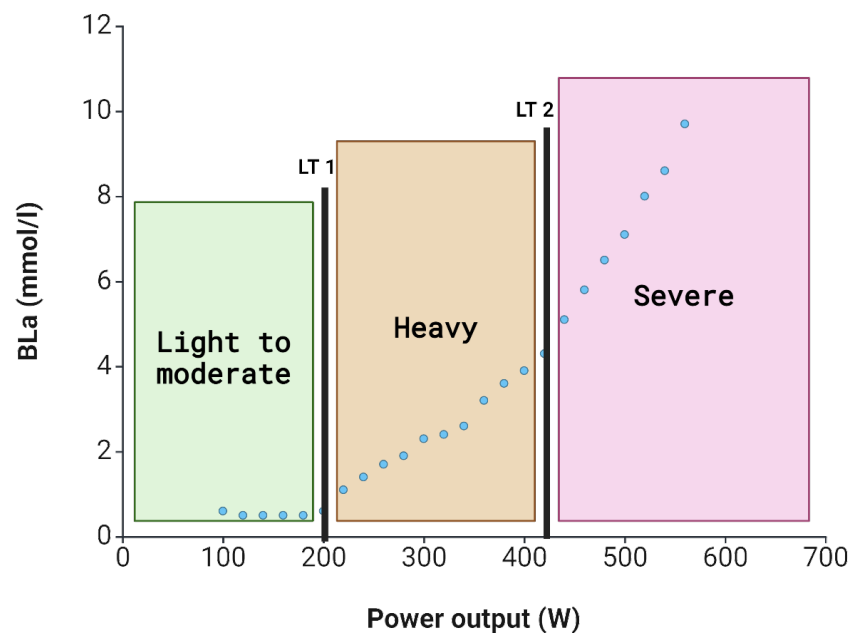


Figure 4. Training intensity distribution

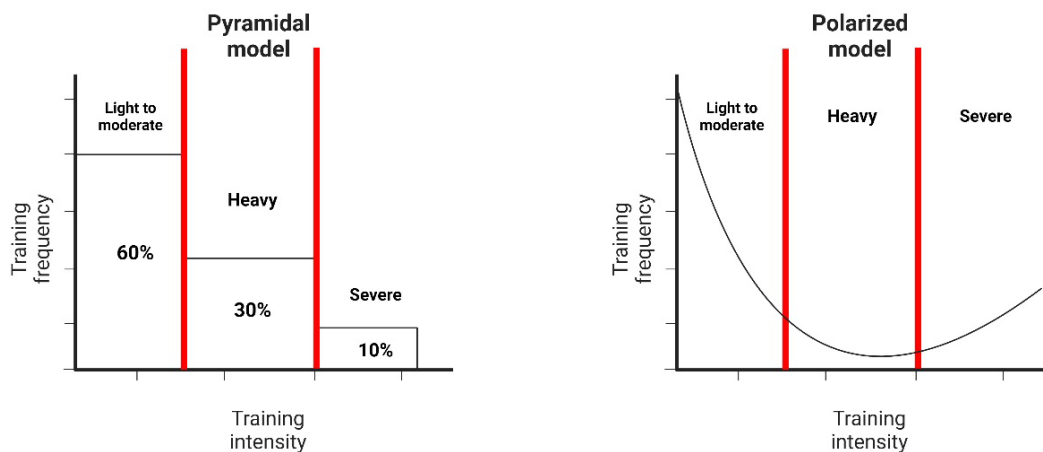


Figure 5. Models of intensity distribution

An important advance in exercise science and in general in sport has been the introduction of training periodization at the start of the 20th century, which by the 1960s was well developed, especially in the USSR (44). This training method tied well with the newly developed idea of intensity distribution in the 1980s and from this emerged the zonal models of intensity distribution. Although the concept was adopted earlier by some running coaches, it was not based on physiological metrics, but on pace, speed or 'feel'. The zonal distribution follows closely the model established by Skinner – three zones, where the transitions are marked by two thresholds, regardless of whether lactate or gas exchange is used to determine them. Although models with up to seven zones exist, the one most firmly based on detectable physiological phenomena is the three-zone model, hence the zones that are described later always refer to it. The first zone relates to light or moderate exercise intensity, which is often quantified as a percentage of VO₂max, heart rate, ventilation, blood lactate level, etc. The second zone between the two thresholds signifies a heavy intensity domain, and the third zone above the second threshold is termed as the severe intensity domain (42). These models of intensity distribution have grown to be very important in training of elite athletes, especially when talking about endurance sports. Virtually all professional athletes train according to some model and three stand out and are most commonly used: the pyramidal, polarised and threshold (45, 46). The names of the models refer to the amount of training sessions performed at the three intensity levels. A pyramidal model includes a big percentage of training designated to zone 1, smaller to zone 2 and the least amount to zone 3. On the contrary a polarised model prescribes training mostly between zone 1 and 3 in an

80:20 ratio and none in zone 2 (46). The use of these two models has been more popular since the 1990s and 2000s, but a newer version in the face of the threshold model has emerged in the last 15 years. It consists of a larger amount of training dedicated to zone 2 between the two lactate thresholds, with training sessions termed 'threshold', which are notorious with the need for rigorous on-field lactate testing (47). This method was popularised mainly owing to the achievements of the Ingebrigtsen brothers – European, world and Olympic champions in middle and long distance running, however it is rarely adopted as a whole, even by them, since it brings a large training load and injury risk (5). Rather, some training elements and sessions are incorporated into the pyramidal model such as 'double threshold' sessions and active lactate testing during these specific sessions. These techniques have shown a profound effect on performance, although anecdotal, in other athletes such as the current Olympic champion in triathlon Kristian Blummenfelt (6, 48).

The scientific literature is equivocal on the most effective model, when looking at performance results. There is conflicting evidence supporting the use of the polarised model, but also such for the pyramidal model, while the threshold model as mentioned is rarely used, so direct evidence is rather lacking (46, 47, 49). Nonetheless, the above examples of athletes are in favour of using a key quality of the threshold approach – lactate-guided interval training, as an emerging novelty in endurance sports (48).

Summary

Lactate metabolism has been a topic considered settled for a long time, but a recent revival in the interest of its production and metabolic fate has sparked a debate. It is now

appreciated that it is produced in fully aerobic conditions, which depends on the metabolic rate of glycolysis, it can be readily oxidised by different tissues for ATP production, it is a major source for gluconeogenesis and last but not least it has important actions as a signaling molecule. All those qualities of lactate are appreciated by exercise scientists, who have sought and developed ways to exploit them in favour of athletes. Incorporating lactate measurements in lab testing and training for endurance sports has significantly elevated the field and yielded immense performances across various disciplines ranging from 400 metres to the marathon in running, but also in cycling, triathlon, etc. Lactate-based training is now in its bloom and it's clear that it has a beneficial effect on training and performance in the short term. What is an interesting topic for future research are the long-term effects that such training could have on athletes, especially on the cardiovascular system and metabolism.

References

1. Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z, Gladden LB. Lactate metabolism: historical context, prior misinterpretations, and current understanding. *Eur J Appl Physiol*. 2018 Apr;118(4):691–728.
2. Wasserman K, McIlroy MB. Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *Am J Cardiol*. 1964 Dec;14(6):844–52.
3. Brooks GA. Current concepts in lactate exchange. *Med Sci Sports Exerc*. 1991 Aug 1;23(8):895–906.
4. Kindermann W, Simon G, Keul J. The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur J Appl Physiol*. 1979 Sep 1;42(1):25–34.
5. Tjelta LI. Three Norwegian brothers all European 1500 m champions: What is the secret? *Int J Sports Sci Coach*. 2019 Oct 1;14(5):694–700.
6. Cejuela R, Sellés-Pérez S. Road to Tokyo 2020 Olympic Games: Training Characteristics of a World Class Male Triathlete. *Front Physiol* [Internet]. 2022 Apr 20 [cited 2024 Mar 31];13. Available from: <https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2022.835705/full>
7. San-Millán I, Stefanoni D, Martinez JL, Hansen KC, D'Alessandro A, Nemkov T. Metabolomics of Endurance Capacity in World Tour Professional Cyclists. *Front Physiol* [Internet]. 2020 Jun 5 [cited 2024 Mar 31];11. Available from: <https://www.frontiersin.org/journals/physiology/articles/10.3389/fphys.2020.00578/full>
8. Li X, Yang Y, Zhang B, Lin X, Fu X, An Y, et al. Lactate metabolism in human health and disease. *Signal Transduct Target Ther*. 2022 Sep 1;7(1):305.
9. Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol-Regul Integr Comp Physiol*. 2004 Sep;287(3):R502–16.
10. Robergs R, O'Malley B, Torrens S, Siegler J. The missing hydrogen ion, part-1: Historical precedents vs. fundamental concepts. *Sports Med Health Sci*. 2023 Nov 13;5.
11. Robergs RA, McNulty CR, Minett GM, Holland J, Trajano G. Lactate, not Lactic Acid, is Produced by Cellular Cytosolic Energy Catabolism. *Physiology*. 2018 Jan;33(1):10–2.
12. Brooks GA. The lactate shuttle during exercise and recovery. *Med Sci Sports Exerc*. 1986 Jun;18(3):360–8.
13. Brooks GA. Lactate as a fulcrum of metabolism. *Redox Biol*. 2020 Aug;35:101454.
14. Brooks GA. Lactate shuttles in Nature. *Biochem Soc Trans*. 2002;30.
15. Felmlee MA, Jones RS, Rodriguez-Cruz V, Follman KE, Morris ME. Monocarboxylate Transporters (SLC16): Function, Regulation, and Role in Health and Disease. *Pharmacol Rev*. 2020 Apr;72(2):466–85.
16. Ga B. The Science and Translation of Lactate Shuttle Theory. *Cell Metab* [Internet]. 2018 Apr 3 [cited 2024 Apr 5];27(4). Available from: <https://pubmed.ncbi.nlm.nih.gov/29617642/>
17. Van Hall G. Lactate kinetics in human tissues at rest and during exercise. *Acta Physiol*. 2010 Aug;199(4):499–508.
18. Brooks GA. Mammalian fuel utilization during sustained exercise. *Comp Biochem Physiol B Biochem Mol Biol*. 1998 May;120(1):89–107.
19. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. *J Physiol*. 2004 Jul;558(1):5–30.
20. Wang Y, Kwon H, Su X, Wondisford FE. Glycerol not lactate is the major net carbon source for gluconeogenesis in mice during both short and prolonged fasting. *Mol Metab*. 2020 Jan 1;31:36–44.
21. Brooks GA, Osmond AD, Arevalo JA, Duong JJ, Curl CC, Moreno-Santillan DD, et al. Lactate as a myokine and exerkine: drivers and signals of physiology and metabolism. *J Appl Physiol*. 2023 Mar 1;134(3):529–48.
22. San-Millán I, Brooks GA. Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis*. 2016 Dec 17;bgw127.
23. Lundin G, Ström G. The Concentration of Blood Lactic Acid in Man during Muscular Work in

- Relation to the Partial Pressure of Oxygen of the Inspired Air. *Acta Physiol Scand*. 1947 Aug;13(3):253–66.
24. Poole DC, Rossiter HB, Brooks GA, Gladden LB. The anaerobic threshold: 50+ years of controversy. *J Physiol*. 2021 Feb;599(3):737–67.
25. Stallknecht B, Vissing J, Galbo H. Lactate production and clearance in exercise. Effects of training. A mini-review. *Scand J Med Sci Sports*. 1998 Jun;8(3):127–31.
26. Emhoff CAW, Messonnier LA. Concepts of Lactate Metabolic Clearance Rate and Lactate Clamp for Metabolic Inquiry: A Mini-Review. *Nutrients*. 2023 Jul 20;15(14):3213.
27. Wan JJ, Qin Z, Wang PY, Sun Y, Liu X. Muscle fatigue: General understanding and treatment. *Exp Mol Med*. 2017;49(10).
28. Owles WH. Alterations in the lactic acid content of the blood as a result of light exercise, and associated changes in the co₂-combining power of the blood and in the alveolar co₂ pressure. *J Physiol*. 1930 Apr 14;69(2):214–37.
29. Rammal K, Strom G. The rate of lactate utilization in man during work and at rest. *Acta Physiol Scand*. 1949 Apr 14;17(4):452–6.
30. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol Bethesda Md* 1985. 1986 Jun;60(6):2020–7.
31. Wasserman K, Whipp BJ, Koys SN, Beaver WL. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol*. 1973 Aug;35(2):236–43.
32. Reybrouck T, Weymans M, Ghesquiere J, Van Gerven D, Stijns H. Ventilatory threshold during treadmill exercise in kindergarten children. *Eur J Appl Physiol*. 1982;50(1):79–85.
33. Faude O, Kindermann W, Meyer T. Lactate Threshold Concepts: How Valid are They? *Sports Med*. 2009 May;39(6):469–90.
34. Shimojo N, Naka K, Nakajima C, Yoshikawa C, Okuda K, Okada K. Test-strip method for measuring lactate in whole blood. *Clin Chem*. 1989 Sep;35(9):1992–4.
35. Shimojo N, Naka K, Uenoyama H, Hamamoto K, Yoshioka K, Okada K. Electrochemical assay system with single-use electrode strip for measuring lactate in whole blood. *Clin Chem*. 1993 Nov;39(11 Pt 1):2312–4.
36. Heuberger JAAC, Gal P, Stuurman FE, De Muinck Keizer WAS, Mejia Miranda Y, Cohen AF. Repeatability and predictive value of lactate threshold concepts in endurance sports. Mourout L, editor. *PLOS ONE*. 2018 Nov 14;13(11):e0206846.
37. Heck H, Wackerhage H. The origin of the maximal lactate steady state (MLSS). *BMC Sports Sci Med Rehabil*. 2024 Feb 5;16(1):36.
38. Sjödin B, Jacobs I, Svedenhag J. Changes in onset of blood lactate accumulation (OBLA) and muscle enzymes after training at OBLA. *Eur J Appl Physiol*. 1982;49(1):45–57.
39. Heck H, Mader A, Hess G, Mücke S, Müller R, Hollmann W. Justification of the 4-mmol/l Lactate Threshold. *Int J Sports Med*. 1985 Jun;06(03):117–30.
40. Cheng B, Kuipers H, Snyder A, Keizer H, Jeukendrup A, Hesselink M. A New Approach for the Determination of Ventilatory and Lactate Thresholds. *Int J Sports Med*. 1992 Oct;13(07):518–22.
41. Bishop D, Jenkins DG, Mackinnon LT. The relationship between plasma lactate parameters, Wpeak and 1-h cycling performance in women. *Med Sci Sports Exerc*. 1998 Aug;30(8):1270–5.
42. Skinner JS, Mclellan TH. The Transition from Aerobic to Anaerobic Metabolism. *Res Q Exerc Sport*. 1980 Mar;51(1):234–48.
43. Binder RK, Wonisch M, Corra U, Cohen-Solal A, Vanhees L, Saner H, et al. Methodological approach to the first and second lactate threshold in incremental cardiopulmonary exercise testing. *Eur J Cardiovasc Prev Rehabil*. 2008 Dec;15(6):726–34.
44. Pedemonte J. Foundations of training periodization Part I: historical outline. *Strength Cond J*. 1986 Jun;8(3):62.
45. Seiler KS, Kjerland GØ. Quantifying training intensity distribution in elite endurance athletes: is there evidence for an “optimal” distribution? *Scand J Med Sci Sports*. 2006 Feb;16(1):49–56.
46. Kenneally M, Casado A, Santos-Concejero J. The Effect of Periodization and Training Intensity Distribution on Middle- and Long-Distance Running Performance: A Systematic Review. *Int J Sports Physiol Perform*. 2018 Oct 1;13(9):1114–21.
47. Casado A, González-Mohino F, González-Ravé JM, Foster C. Training Periodization, Methods, Intensity Distribution, and Volume in Highly Trained and Elite Distance Runners: A Systematic Review. *Int J Sports Physiol Perform*. 2022 Jun 1;17(6):820–33.
48. Casado A, Foster C, Bakken M, Tjelta LI. Does Lactate-Guided Threshold Interval Training within a High-Volume Low-Intensity Approach Represent the “Next Step” in the Evolution of Distance Running Training? *Int J Environ Res Public Health*. 2023 Jan;20(5):3782.
49. Stöggl T, Sperlich B. Polarized training has greater impact on key endurance variables than threshold, high intensity, or high volume training. *Front Physiol [Internet]*. 2014 [cited 2024 May 10];5. Available from: <http://journal.frontiersin.org/article/10.3389/fphys.2014.00033/abstract>

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The seemingly hidden role of right ventricular dysfunction in the pathophysiology of sudden cardiac death among elite athletes

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Introduction: Sudden cardiac death (SCD) has been acknowledged as a significant cause of mortality among apparently healthy elite athletes. Traditionally, much focus has been directed towards the role of the left ventricle and its pathophysiology. Yet, emerging research highlights importance of right ventricular dysfunction in the development of sudden cardiac death. **Aim:** This review aims to explore new findings about the role of right ventricular dysfunction regarding the pathogenesis of sudden cardiac death in elite athletes. **Material and methods:** A search in the PubMed database was performed utilizing the following keywords: sudden cardiac death and elite athletes, sudden cardiac death, and right ventricle. Twenty-five articles were selected. **Results and discussion:** Endurance exercise places a disproportional load on the right ventricle, both physically and metabolically, due to its thinner chamber wall, relative to the left ventricle. This seems to contribute to pro-arrhythmic right ventricular remodeling. Furthermore, right ventricular dysfunction is observed following intense endurance exercise, evidenced further by inversely correlated elevation of biomarkers such as Troponin and B-type natriuretic peptide. Moreover, morphological changes to the right ventricle may not be entirely reversible as opposed to left ventricular changes as seen in athletic heart syndrome. **Conclusion:** Sudden cardiac death is a leading cause of mortality in elite athletes, despite routine screenings and annual medical evaluations. This indicates a gap in our comprehension of the underlying pathogenesis and underlines the need for further research in this area.

Keywords: sudden cardiac death, right ventricular dysfunction, right ventricle, elite athletes, sports medicine

Introduction

Sudden cardiac death (SCD) has been acknowledged as a significant cause of mortality among apparently healthy elite endurance athletes (1). Most common causes in professionals include Hypertrophic Cardiomyopathy (HCM), Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC), and Coronary Artery Anomalies (CAA) (1, 2, 3). Other less frequent causes are Marfan's syndrome, myocarditis, dilated cardiomyopathy, Brugada syndrome, long QT syndromes, and Wolf-Parkinson-White syndrome (2). Engaging in sports isn't the direct cause of death, but it can trigger cardiac arrest in individuals with underlying cardiovascular conditions that make them susceptible to serious ventricular arrhythmias (2, 4, 5). Although infrequent, such events can leave devastating effects on the sports community (6) and often receive substantial media attention in part due to the paradox of

athletes, presumed to be some of the healthiest members of our society, nevertheless being stuck by a condition often associated with a sedentary lifestyle in popular culture. Reported incidence varies vastly among different studies due to differences in inclusion criteria with cases ranging from 1/39,000 to 1/281,000 across all ages, whereas in young athletes 1–2 per 100,000 (1). A consistent finding among all studies is that male athletes have 3 to 5 times higher incidence of SCD than female athletes (17). Furthermore, data from the United Kingdom indicated incidence of SCD in previously screened soccer players from 1 per 14,794 person-years or 6.8 per 100,000 in athletes (1). This undoubtedly questions the validity of pre-participation examination (PPE), introduced back in 1966 in the US and which is nevertheless based on observational data and thus only endorsed rather than mandated. Traditionally, much focus has been directed

towards the role of the left ventricle and its pathophysiology. Yet, emerging research highlights the importance of right ventricular dysfunction in the development of sudden cardiac death (8).

Aim

This review aims to explore new findings regarding the role of right ventricular dysfunction in the pathogenesis of sudden cardiac death in elite athletes.

Material and methods

A search in the PubMed database was performed utilizing the following keywords: sudden cardiac death and elite athletes, sudden cardiac death, and right ventricle. Twenty-five articles were selected, published in the time period from 1988 to 2022.

Results and discussion

Over recent decades, numerous studies have delineated the phenomenon of adaptive remodeling, often dubbed as the “athlete's heart”. Physical activity induces a range of structural, functional, and electrical modifications in the heart, which are natural responses to the increased hemodynamic demands during exercise (9). These changes primarily manifest as cardiac enlargement while maintaining compliance and contractility, thereby facilitating more efficient filling and emptying of larger volumes, as described by Laplace's law. Repeated sessions of vigorous exercise can induce mild cardiac hypertrophy and/or chamber expansion, which typically reverses upon sustained cessation of exercise. The type and intensity of exercise dictate the nature and extent of exercise-induced cardiac remodeling, with the hemodynamic alterations during exercise serving as stimuli for growth and adaptation of the heart chambers. Isometric or static exercises, such as strength training (e.g.,

weightlifting, wrestling), involve brief, intense periods of heightened peripheral vascular resistance with minimal changes in cardiac output. These activities are linked to mild concentric hypertrophy and a normal to slightly enlarged left atrium, primarily attributed to the addition of sarcomeres within cardiomyocytes. In contrast, prolonged isotonic or dynamic aerobic exercise, commonly termed endurance exercise (e.g., long-distance running, cycling, rowing, or swimming), necessitates sustained increases in cardiac output while typically exhibiting normal or reduced peripheral vascular resistance. Endurance exercise fosters eccentric left ventricular hypertrophy, right ventricular dilation, and enlargement of both atria (8, 9).

As previously mentioned, alterations in the dimensions and hemodynamics of the right ventricle (RV) and pulmonary circulation can be observed, posing challenges in evaluating athletes due to the similarities between RV parameters seen in both physiological and pathological states. Therefore, assessing RV and pulmonary circulation during exercise is crucial for understanding the underlying physiological mechanisms driving these changes (8).

Applying the basic principle of the Laplace relationship, Douglas et al. observed that during exercise, the RV experienced greater increases in both pressures and volumes compared to the left ventricle (LV), with a relatively smaller increase in wall thickness. Consequently, estimates of RV wall stress surged by 125% during exercise, in stark contrast to the modest 14% rise in LV wall stress (10).

Hence, it could be argued that the stress, workload, and metabolic requirements imposed on the RV during exercise are considerably higher compared to those of the LV (Fig. 1). Multiple studies have indicated that

vigorous, prolonged exercise leads to a noticeable decline in right ventricular (RV) function, while left ventricular (LV) function remains relatively unaffected (11, 12, 13, 14). Research on endurance athletes post-competitive events across various disciplines and durations has revealed signs of acute myocardial injury, indicated by transient increases in biomarkers such as Troponin and B-type natriuretic peptide (15). A meta-analysis suggested that around 50% of participants in endurance exercise events experience elevated levels of cardiac troponin (16). While these cardiac enzyme levels typically return to normal within a few days post-exercise, there remains a debate regarding their origin, mechanisms, and significance. However, it appears that changes in right ventricular (RV) function are notably more pronounced and may offer a more plausible explanation for the elevation in biomarkers. As discussed earlier, studies on

post-endurance exercise consistently demonstrate greater declines in RV function compared to the left ventricle (LV).

One possible harmful outcome of cardiac injury is the development of chronic scar tissue or fibrosis, which can impair cardiac function and/or lead to arrhythmias (Fig. 1). The most promising noninvasive method for detecting fibrosis involves utilizing cardiac magnetic resonance imaging (MRI) with gadolinium contrast. This technique, known as delayed gadolinium enhancement (DGE), has been examined in small athlete cohorts. While DGE seems to be lacking in athletes with limited training backgrounds (15, 17, 18) recent research indicates its presence in 12–50% of highly trained veteran athletes (19, 20). La Gerche et al. discovered DGE in 5 out of 39 well-trained endurance athletes, noting that those with DGE had a longer training history and larger cardiac dimensions, especially in the right ventricle (21).

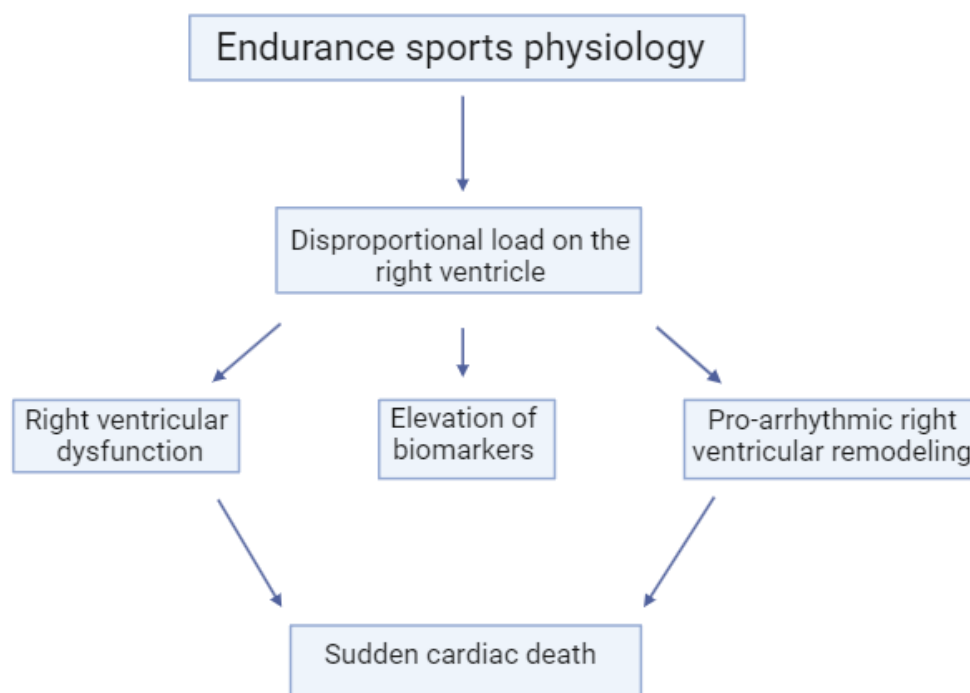


Figure 1. Intense endurance exercise disproportionately affects the RV, leading to structural and functional changes, including pro-arrhythmic remodeling and acute myocardial injury

The implications of these findings are significant. Firstly, changes in right heart morphology and function observed in athletes can affect resting electrocardiography (ECG) patterns, including common occurrences of complete and incomplete right bundle branch block. These ECG findings can overlap with primary conduction disturbances and cardiomyopathies, particularly arrhythmogenic right ventricular cardiomyopathy (ARVC). Secondly, there is compelling evidence linking long-standing endurance training with certain cardiac arrhythmias. Studies consistently show an increased prevalence of atrial fibrillation in middle-aged and older endurance athletes compared to non-athletic individuals (22, 23). While ventricular ectopic beats are common among athletes, they are generally considered benign if underlying cardiac disease is ruled out (24). However, some research, such as that by Heidbuchel et al., has shown a high incidence of major arrhythmic events, including sudden cardiac death, among athletes with frequent ventricular ectopy or ventricular tachycardia (5). These arrhythmias often originate from the right ventricle (RV) and are associated with functional and/or structural changes in the RV. The extent to which years of intense endurance exercise promote ventricular arrhythmias remains uncertain, as does the role of sports practice itself versus underlying familial predispositions like ARVC (25). These findings underscore the importance of careful cardiac evaluation and monitoring in athletes, particularly those engaged in endurance sports, to detect and manage potential arrhythmias and associated risks.

Conclusion

Recent research highlights the significant role of right ventricular dysfunction in sudden cardiac death among elite athletes,

challenging the traditional focus on the left ventricle. Intense endurance exercise disproportionately affects the RV, leading to structural and functional changes, including pro-arrhythmic remodeling and acute myocardial injury. Delayed gadolinium enhancement MRI reveals fibrosis in highly trained athletes, indicating potential long-term consequences. Furthermore, endurance training is associated with cardiac arrhythmias, emphasizing the need for comprehensive cardiac evaluation, especially in endurance athletes. While some arrhythmias may be benign, those originating from the RV can pose serious risks, including SCD. Further research is needed to understand the relationship between exercise and arrhythmias versus underlying predispositions like ARVC.

References

1. Hajduczuk AG, Ruge M, Emery MS. Risk Factors for Sudden Death in Athletes, Is There a Role for Screening? *Current Cardiovascular Risk Reports* [Internet]. 2022 [cited 2022 Nov 7];16(10):97–109. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC9251040/
2. Davogustto G, Higgins JP. Sudden Cardiac Death in the Soccer Field: A Retrospective Study in Young Soccer Players From 2000 to 2013. *The Physician and Sportsmedicine*. 2014 Nov 1;42(4):20–9.
3. Tsang DC, Link MS. Sudden Cardiac Death in Athletes. *Texas Heart Institute Journal* [Internet]. 2021 Oct 15;48(4). Available from: <https://meridian.allenpress.com/thij/article/48/4/e207513/472196/Sudden-Cardiac-Death-in-Athletes>
4. Gerche AL, Schmied CM. Atrial fibrillation in athletes and the interplay between exercise and health. *European Heart Journal*. 2013 Jul 24;34(47):3599–602.
5. Heidbüchel H. High prevalence of right ventricular involvement in endurance athletes with ventricular arrhythmias Role of an electrophysiologic study in risk stratification. *European Heart Journal* [Internet]. 2003 Aug [cited 2019 Nov 29];24(16):1473–80. Available from: www.natap.org/2011/newsUpdates/EurHeartHeidbchel147380.pdf
6. D'Ascenzi F, Valentini F, Pistoresi S, Frascaro F, Piu P, Cavigli L, et al. Causes of sudden cardiac death in young athletes and non-athletes: systematic review and meta-analysis. *Trends in Cardiovascular Medicine*. 2021 Jun;32(5).
7. 1.Emery MS, Kovacs RJ. Sudden Cardiac Death in Athletes. *JACC: Heart Failure* [Internet]. 2018 Jan;

- 6(1):30–40. Available from: <http://heartfailure.onlinejacc.org/content/6/1/30>
8. D'Andrea A, Morello A, Iacono A, Scarafile R, Cocchia R, Riegler L, et al. Right ventricular changes in highly trained athletes: Between physiology and pathophysiology. *Journal of Cardiovascular Echography* [Internet]. 2015;25(4):97. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC5353418/
9. Fulghum K, Hill BG. Metabolic Mechanisms of Exercise-Induced Cardiac Remodeling. *Frontiers in Cardiovascular Medicine*. 2018 Sep 11;5.
10. Douglas PS, O'Toole ML, Miller WD, Douglas B, Reichel N. Different effects of prolonged exercise on the right and left ventricles. *Journal of the American College of Cardiology*. 1990 Jan 1;15(1):64–9.
11. Trivax JE, Franklin BA, Goldstein JA, Chinaiyan KM, Gallagher MJ, deJong AT, et al. Acute cardiac effects of marathon running. *Journal of Applied Physiology*. 2010 May;108(5):1148–53.
12. Neilan TG, Yoerger DM, Douglas PS, Marshall JE, Halpern EF, Lawlor D, et al. Persistent and reversible cardiac dysfunction among amateur marathon runners. *European Heart Journal*. 2006 Mar 22;27(9):1079–84.
13. Mousavi N, Czarnecki A, Kumar K, Nazanin Fallah-Rad, Lytwyn M, Han SY, et al. Relation of Biomarkers and Cardiac Magnetic Resonance Imaging After Marathon Running. *American Journal of Cardiology*. 2009 May 1;103(10):1467–72.
14. La Gerche A, Connelly KA, Mooney DJ, MacIsaac AI, Prior DL. Biochemical and functional abnormalities of left and right ventricular function after ultra-endurance exercise. *Heart*. 2008 Jul 1;94(7):860–6.
15. Shave R, Baggish A, George K, Wood M, Scharhag J, Whyte G, et al. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. *Journal of the American College of Cardiology* [Internet]. 2010 Jul 13;56(3):169–76. Available from: <https://pubmed.ncbi.nlm.nih.gov/20620736/>
16. SHAVE R, GEORGE KP, ATKINSON G, HART E, MIDDLETON N, WHYTE G, et al. Exercise-Induced Cardiac Troponin T Release. *Medicine & Science in Sports & Exercise*. 2007 Dec;39(12):2099–106.
17. Antonio Dello Russo, Pieroni M, Santangeli P, Bartoletti S, Casella M, Pelargonio G, et al. Concealed cardiomyopathies in competitive athletes with ventricular arrhythmias and an apparently normal heart: role of cardiac electroanatomical mapping and biopsy. *Heart Rhythm*. 2011 Dec 1;8(12):1915–22.
18. Galderisi M, Cardim N, D'Andrea A, Bruder O, Cosyns B, Davin L, et al. The multi-modality cardiac imaging approach to the Athlete's heart: an expert consensus of the European Association of Cardiovascular Imaging. *European Heart Journal Cardiovascular Imaging* [Internet]. 2015 Apr 1;16(4):353. Available from: <https://pubmed.ncbi.nlm.nih.gov/25681828/>
19. Pollak SJ, McMillan SA, Knopff WD, Wharff R, Yoganathan AP, Felner JM. Cardiac evaluation of women distance runners by echocardiographic color Doppler flow mapping. *Journal of the American College of Cardiology* [Internet]. 1988 Jan 1 [cited 2024 May 12];11(1):89–93. Available from: <https://pubmed.ncbi.nlm.nih.gov/2961794/>
20. Breuckmann F, Möhlenkamp S, Nassenstein K, Lehmann N, Ladd S, Schmermund A, et al. Myocardial Late Gadolinium Enhancement: Prevalence, Pattern, and Prognostic Relevance in Marathon Runners. *Radiology*. 2009 Apr;251(1):50–7.
21. La Gerche A, MacIsaac AI, Burns AT, Mooney DJ, Inder WJ, Voigt JU, et al. Pulmonary transit of agitated contrast is associated with enhanced pulmonary vascular reserve and right ventricular function during exercise. *Journal of Applied Physiology*. 2010 Nov;109(5):1307–17.
22. Karjalainen J, Kujala UM, Kaprio J, Sarna S, Viitasalo M. Lone atrial fibrillation in vigorously exercising middle aged men: case-control study. *BMJ*. 1998 Jun 13;316(7147):1784–5.
23. Grimsby J, Grundvold I, Maehlum S, Arnesen H. High prevalence of atrial fibrillation in long-term endurance cross-country skiers: echocardiographic findings and possible predictors – a 28–30 years follow-up study. *European Journal of Cardiovascular Prevention & Rehabilitation*. 2010 Feb;17(1):100–5.
24. Biffi A, Maron BJ, Verdile L, Fernando F, Spataro A, Marcello G, et al. Impact of physical deconditioning on ventricular tachyarrhythmias in trained athletes. *Journal of the American College of Cardiology*. 2004 Sep;44(5):1053–8.
25. La Gerche A. Defining the interaction between exercise and arrhythmogenic right ventricular cardiomyopathy. *European Journal of Heart Failure*. 2014 Dec 31;17(2):128–31.

Anthropological comparison of dimensions of dental crowns between Bulgarian and Indian population

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Introduction: Dental profile consists of specific individual characteristics related to the teeth and their size. They can help in estimation of age, sex, race, socio-economic status, personal habits, oral and systemic health, occupation and dietary status of the person. Variability observed in the human dentition provides a theoretical basis for the individualization of human dentition. Numerous factors can contribute to variation in tooth size but probably the combination between genetic factors and environmental influences leads to the differences between populations. The **aim** of the present study is to evaluate the variations of dental dimensions between Bulgarians and Indians. **Materials and methods:** The study included 232 Bulgarians aged 20–40 years. Vestibulolingual and mesiodistal dimensions of teeth were measured by Dentistry Sliding Vernier Caliper and analyzed with SPSS 23.0. **Results:** We found significant differences in mesiodistal dimensions of maxillary canines, premolars and molars and mandibular incisors and premolars between Bulgarians and Indians. Some of them were with high degree of significance. **Conclusion:** Our results revealed that odontometric dimensions vary in different population and therefore it is necessary to determine specific population values in order to make identification possible.

Keywords: dental dimensions, dental profile, population-specific, Bulgarians

Introduction

Dental profile consists of specific individual characteristics related to the teeth and their size. They can help in estimation of age, sex, race, socio-economic status, personal habits, oral and systemic health, occupation and dietary status of the person [1]. Variability observed in the human dentition provides a theoretical basis for the individualization of human dentition [2]. Different dental traits, such as crown dimensions, tooth shape, cusp number, groove and fissure patterns can provide evidence about the nature and extent of diversity between human populations [3]. Examples of ethnic differences and geographic variability in tooth size have been documented. Numerous factors can contribute to variation in tooth size but probably the combination between genetic factors and environmental influences leads to the differences between populations [4].

A synthesis of data on dental dimensions from different population worldwide has indicated that Western Eurasian population tend to have the smallest teeth while Australians, sub-Saharan Africans and Native

Americans tend to have the largest teeth. East and Southeast Asian population were found to be intermediate in tooth size between these groups. Hanishara observed that this was due to the impact of agriculture [5]. Masticator forces, non-chewing parafunctional activities, use of teeth as tools, nature of the diet can also contribute to variations in dental size between different populations [6]. A tough fibrous and abrasive food requires prolonged mastication so populations relying on plant food have larger teeth than these eating meat. Environmental influences can affect the dentition during prenatal and postnatal periods and depending on the time of the effect a variety of phenotypic variations may occur [7]. In accordance with other researchers [8] we think that variations in crown size between different populations are affected by interaction between genetic and environmental factors. Standards for skeletal identification vary among different populations and may not be applicable from one population to another. There are no odontometric standards for Bulgarian population.

Materials and methods

The present study included 121 males and 111 females of Bulgarian origin living in South Bulgaria aged 20–40 years. Before starting the study, subjects were informed about the nature of the study and written informed consents were obtained. Patients were included based on the following criteria: presence of complete set of fully erupted and periodontally healthy teeth, presence of non-carious and non-worn teeth, no dental history of any crown restorations or bridges, normal occlusion. Patients with orthognathic surgery or trauma, history or clinical evidence of cleft palate, history or clinical features suggestive of endocranial disorders, metabolic disorders, developmental disorders and history of prolonged illness were excluded.

Vestibulolingual and mesiodistal dimensions of teeth were measured by Dentistry Sliding Vernier Caliper, Ridge Mapping Caliper Type A and Type B. We used the technique of Martin-Saller, 1957, modified by Prof. Yordanov [9]. According to him the mesiodistal dimension is the greatest mesiodistal distance between the contact points of teeth, usually it is in the upper or middle third of coronal height. It is also termed the dental width. The vestibulolingual dimension, also termed as the dental thickness is the greatest dimension between buccal and lingual surfaces of crown, taken at right angle to the plane in which mesiodistal diameter is taken.

The measurements were analyzed with SPSS 23.0 using Student's t-test. The level of statistical significance was set at $P < 0.05$. The degree of significance was considered weak ($P < 0.05$), moderate ($0.01 > P > 0.001$) or high ($P < 0.001$).

Results

1. We found statistically significant differences between the mesiodistal dimensions of maxillary canines and first and

second molars of the **Bulgarian and Indian men** with high degree of significance ($P < 0.001$). The mean values in Bulgarians were higher. We also detected statistically significant differences in the vestibulolingual dimensions of upper incisors and molars between both populations, this time mean values of the Indians were higher (Table 1).

2. Similar differences were found in the mesiodistal dimensions of mandibular lateral incisors and second premolars again with high degree of significance ($P < 0.001$) and in the vestibulolingual dimensions of lower incisors and first premolars. This time mean values in Indians were higher than those in Bulgarians except for the vestibulolingual dimension of first premolar (Table 2).

3. Mesiodistal dimensions of the maxillary canines and first premolars in **Bulgarian women** showed significantly different values than those in **Indian women**. For the canines the degree of significance was high ($P < 0.001$) while for the premolar the degree was weak ($P < 0.05$). Similar differences with high degree of significance were found in the vestibulolingual dimensions of maxillary central incisors, while the degree of significance for the lateral incisors was moderate and for the first molars was weak. Mean values in Bulgarians were lower except for the mesiodistal dimension of the canines (Table 3).

4. We found statistically significant differences between the mesiodistal dimensions of mandibular premolars with moderate degree of significance, statistically significant differences with moderate degree were detected in the vestibulolingual dimensions in lower lateral incisors, canines and second premolars, while first premolars exhibited differences with high degree of significance. The mean values in Bulgarian women were higher for the vestibulolingual dimensions of canines and premolars, the rest were higher in Indian females (Table 4).

Table 1. Comparison of mesiodistal and vestibulolingual dimensions of maxillary teeth between Bulgarian and Indian men

Tooth	Bulgarian males			Indian males			
	N	Mean	SD	N	Mean	SD	P
I11VL	121	6.74	1.18	51	7.26	0.63	0.007
I12VL	121	5.98	0.71	51	6.34	0.47	0.003
C13MD	121	8.72	0.63	51	7.65	0.54	0.001
M16MD	121	10.70	0.67	51	9.96	0.55	0.001
M16VL	121	10.84	0.53	51	11.21	0.62	0.002
M17MD	121	10.00	0.53	51	9.43	0.72	0.001
M17VL	121	10.47	0.55	51	10.89	0.66	0.001

Table 2. Comparison of mesiodistal and vestibulolingual dimensions of mandibular teeth between Bulgarian and Indian men

Tooth	Bulgarian males			Indian males			
	N	Mean	SD	N	Mean	SD	P
I41VL	121	5.60	0.69	51	5.86	0.43	0.029
I42VL	121	5.63	0.69	51	6.10	0.42	0.001
I42MD	121	5.47	0.55	51	5.88	0.41	0.001
P44VL	121	8.70	0.96	51	7.92	0.54	0.001
P45MD	121	6.37	0.82	51	6.82	0.46	0.001

Table 3. Comparison of mesiodistal and vestibulolingual dimensions of maxillary teeth between Bulgarian and Indian women

Tooth	Bulgarian females			Indian females			
	N	Mean	SD	N	Mean	SD	P
I11VL	111	6.58	0.79	51	7.06	0.47	0.001
I12VL	111	5.93	0.63	51	6.27	0.53	0.005
C13MD	111	7.95	0.65	51	7.44	0.40	0.001
P14MD	111	6.49	0.67	51	6.77	0.45	0.017
M16VL	111	10.53	0.59	51	10.85	0.64	0.013

Table 4. Comparison of mesiodistal and vestibulolingual dimensions of mandibular teeth between Bulgarian and Indian women

Tooth	Bulgarian females			Indian females			
	N	Mean	SD	N	Mean	SD	P
I41VL	111	5.83	0.53	51	6.15	0.58	0.008
C43VL	111	7.44	0.85	51	7.03	0.51	0.004
P44VL	111	8.51	0.83	51	7.79	0.50	0.000
P44MD	111	6.47	0.63	51	6.78	0.46	0.009
P45VL	111	8.65	0.72	51	8.28	0.51	0.004
P45MD	111	6.37	0.72	51	6.74	0.48	0.003

Discussion

We found statistically significant differences in 12 odontometric dimensions between Bulgarian and Indian men. Mesiodistal dimensions of maxillary canines and molars were higher in Bulgarians than those in Indians while mesiodistal dimensions in mandibular lateral incisors and second

premolars in Indians were higher than those in Bulgarians. Vestibulolingual dimensions of maxillary incisors and canines and mandibular incisors and first premolars also exhibited statistically significant differences. We found statistically significant differences in 11 dimensions between Bulgarian and Indian women, and these were mesiodistal

dimensions of upper canines and first premolars, lower premolars and vestibulolingual dimensions of maxillary incisors and first premolars and mandibular lateral incisors, canines and premolars. We think that these numerous differences between the two populations are due to environmental factors such as eating habits [10]. Deepak et al found that abrasive food required more continuous mastication hence dental size in these populations were smaller while populations relying more on plant food than meat had larger teeth [11]. Hanishara and Ishida proposed that the smaller tooth dimensions in Western Eurasians populations were related to the lower impact of natural selection on tooth size over the last few millennia, associated with cultural changes in food preparation practices following the adoption of agriculture [2].

Other authors suggested strong genetic contribution in tooth size as well [12]. They have pointed the importance of the role of genetic influences on dental traits and dimensions.

The fact that there were less differences between women than between men and that most of them were in the vestibulolingual dimensions for the women shows that not only odontometric dimensions are population-specific but the degree of sexual dimorphism as well shows variation among different nations.

Conclusion

The present study revealed that odontometric dimensions were population-specific. They can be used for establishing the phylogenetical and biological relationships between populations, for explaining historical, cultural and biological macro and micro-evolutionary processes and thus for

understanding the origin, formation, contacts, migration pathways of the different populations leading to ethnic variation of humanity.

References

1. **Jensen, E., YP. Kai-Jen, CF. Moorrees, SO. Thomsen.** Mesiodistal crown diameters of the deciduous and permanent teeth in individuals. *J Dent Res.*; **36**(1), 1957 Feb, 39-47.
2. **Hanihara, T., H. Ishida.** Metric dental variation of major human populations. *Am J Phys Anthropol*; **128**(2), 2005, 287-98
3. **Moorrees, C.,** The dentition of the growing child: A longitudinal study of dental development between 3 and 18 years of age. Harvard University Press, Cambridge, vol. **138**, issue 4, 1959, 496-496
4. **Brook, AH., J. Jernvall, RN. Smith, TE. Hughes, GC. Townsend.** The dentition: the outcomes of morphogenesis leading to variations of tooth number, size and shape. *Aust Dent J.*; **59**, 2014, 131-42.
5. **Hanihara, K.,** Distances between Australian aborigines and certain other populations based on dental measurements *Journal of Human Evolution*, Volume **6**, Issue 4, May 1977, Pages 403-418
6. **Brook, AH.** A unifying aetiological explanation for anomalies of human tooth number and size. *Arch Oral Biol.*; **29**(5), 1984, 373-8.
7. **Taji, S., T. Hughes, J. Rogers, G. Townsend.** Localised enamel hypoplasia of human deciduous canines: Genotype or environment? *Australian dental journal.*, **45**, 2000, 83-90.
8. **Dempsey, PJ., GC. Townsend,** Genetic and environmental contributions to variation in human tooth size, *Heredity*, Volume **86**, Issue 6, 2001 June, 685-693
9. **Yordanov, Y., K. Uzunov, H. Fakih. Manual in anatomy and anthropology for dentists.** 1st edition, Artgraf, Sofia, 2012, 248-258 [in Bulgarian].
10. **Ates, M., F. Karaman, MY. Iscan, TL. Erdem,** Sexual differences in Turkish dentition, *Leg. Med. Tokyo*, **8**, 2006, 288-292
11. **Deepak, V., SN. Goryawala, Y. Reddy, RJ. Chhabra, Nandaprasad, NK. Shah,** Assessment of Ethnicity in Indian Population using Tooth Crown Metric Dental Traits, *JIOH*; **7**(9), 2015, 83-87
12. **Townsend, GC., NG. Martin,** Fitting Genetic Models to Carabelli Trait Data in South Australian Twins. *Journal of Dental Research*, **71**(2), 1992, 403-409.

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Cognitive impairment in early stages of Parkinson's disease

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Introduction: Parkinson's disease (PD) is a common neurodegenerative condition characterized by motor and nonmotor symptoms. Among non-motor features the most significantly contributing to the patients' disability are cognitive decline and dementia. **Aim:** To evaluate the cognitive functions in newly diagnosed, treatment naïve patients with early PD. **Materials and methods:** The study comprised 28 newly diagnosed patients with PD according to the Movement Disorder Society – Parkinson's Disease (2015) criteria. The severity of motor impairment was evaluated by Unified Parkinson's Disease Rating Scale (UPDRS). The cognitive functions were assessed by Montreal Cognitive Assessment (MOCA) and Mini Mental State Examination (MMSE) tests. **Results:** More than 2/3 of the patients showed abnormally low score on cognitive tests (92.9% on MOCA and 78.6% on MMSE). Moderate negative correlation was found between MOCA results and age of the patients ($r_{xy} = 0.553$, $p = 0.002$), and MOCA results and the age at the onset of the disease ($r_{xy} = 0.528$, $p = 0.004$). No significant correlations were found between the MMSE score, age of the patients, the age at disease onset and the duration of the disease. **Conclusion:** A large part of patients with newly diagnosed PD have different degree of cognitive dysfunctions. The age of the patients and the age at the disease onset can contribute to increased cognitive impairment in the early stages of Parkinson's disease.

Keywords: cognitive impairment, Parkinson's disease, non-motor symptoms, dementia

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting more than 1% of the population over the age of 65¹. It is characterized by motor (tremor, rigidity, bradykinesia and postural instability) and non-motor (cognitive decline, depression, psychosis, autonomic disorders) symptoms. Among the non-motor symptoms of greatest importance for patients' disability is cognitive deficit, progressing over time to dementia². Mild cognitive impairment occurs in about 20% of PD cases at diagnosis³. In the course of the disease, it can affect up to 60%³ of patients. Dementia is described in 30 to 40% of patients with PD, and can reach 80% with increasing age and duration of the disease^{3,4}. Cognitive deficit occurs up to six times more often in individuals with PD compared to the healthy population¹ and is the subject of increasing scientific interest due to its determining importance for the social adaptation and quality of life of the patients.

Aim

To assess cognitive function in newly diagnosed, drug naïve patients with early PD.

Materials and methods

Patients:

The study included 28 patients selected by the Expert Committee for diagnosis and treatment of PD at UMHAT "Kaspela", in accordance with the following criteria:

Inclusion criteria:

1. Age 18–80 years;
2. Newly diagnosed patients with Parkinson's disease according to the MDS-PD criteria;
3. Written informed consent to participate in the study.

Exclusion criteria:

1. Patients with decompensated heart, lung, kidney, endocrine or liver diseases;
2. Patients with sleep apnea;
3. Patients with mental diseases (schizophrenia, bipolar affective disorder);
4. Pregnancy;
5. Patients abusing alcohol, narcotic substances and medications;
6. Inability to declare informed consent.

Methods:

1. Clinical methods: medical history, assessment of neurological status, determination of the degree of disability using the

Unified Parkinson's Disease Rating Scale (UPDRS).

2. Neuropsychological tests to study the state of cognitive functions:

- Montreal Cognitive Assessment (MOCA),
- The Mini-Mental State Exam (MMSE),
- Isaac's Test

3. Statistical methods.

Results

The study included 28 newly diagnosed patients according to MDS-PD criteria. Demographic and clinical characteristics of the study cohort are presented in Table 1. Results of the MOCA and MMSE cognitive tests are presented in Table 2. Only 7.1% (n = 2) demonstrated normal score on the MOCA. The remaining 92.9% (n = 26) have varying degrees of cognitive deficit. On the MMSE test, 21.4% (n = 6) had a normal results, and 78.6% (n = 22) had an abnormally low score. The distribution of patients according to the severity of cognitive deficit assessed by MMSE is presented in Figure 1. Correlation analysis found a moderate negative correlation between MOCA scores and patients' age ($r_{xy} = 0.553$, $p = 0.002$), as well as with age at onset of complaints ($r_{xy} = 0.528$, $p = 0.004$). No statistically significant correlation was found between MMSE, patient age, age at onset and disease duration.

Table 1. Demographic and clinical characteristics of the studied contingent

Characteristics	Patients
Sex (number/%)	
women	19/67.9%
men	9/32.1%
Age	
mean $\pm$ SD (years)	69.25 $\pm$ 7.69
Age at onset of complaints	
mean $\pm$ SD (years)	68.04 $\pm$ 7.24
Duration of the complaints before diagnosis	
mean $\pm$ SD (months)	14.9 $\pm$ 11.4
Total UPDRS score	
mean $\pm$ SD (points)	37.39 $\pm$ 14.28

Table 2. Cognitive assessment

Test	Mean $\pm$ SD	minimum	maximum
MOCA	20.54 $\pm$ 3.99	11	28
MMSE	25.29 $\pm$ 2.65	18	29

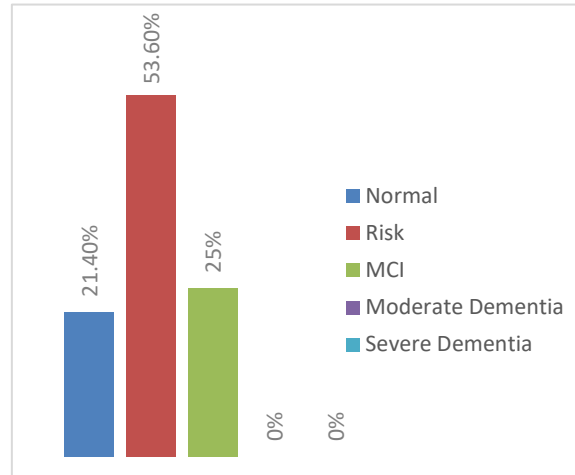


Figure 1. Distribution of patients according to the severity of cognitive deficit assessed by MMSE

Discussion

The present study reported cognitive deficits in more than two-thirds of the patients. Mild cognitive impairment was observed in 25% of the patients' population according to the MMSE. The same data were found by Hemphill et al., 2023. Close results were presented by Litvan et al., 2011 (26.7%)⁹, and Jaramillo-Jimenez et al., 2023 (36%)¹⁰. The reasons for these differences should be sought in the heterogeneity of the studied contingent (accompanying diseases, the age at the onset of complaints, etc.), the different neuropsychological tests used for the assessment of cognitive functions and/or in different criteria for defining cognitive decline. The occurrence of cognitive deficits in two thirds of our patients corresponds to the cognitive impairments recorded by Wan et al., 2023 in 75.4% of cases with early PD¹¹.

Patients' age and age at onset of the disease are factors associated with an increased risk of cognitive decline in early PD, demonstrated by negative relationship between

MOCA and patients' age, as well as between MOCA and age at onset of complaints.

The factors that determine the development of cognitive disorders in PD are the subject of numerous studies, but there are still a number of unclear details and conflicting opinions in the scientific literature. Most authors point to attention, executive functions, information processing speed and verbal fluency as more frequently affected cognitive spheres in PD^{5,6,7}.

Experimental and clinical studies demonstrate a substantial involvement of the loss of nigrostriatal, mesolimbic and mesocorticolimbic dopaminergic neurons in cognitive functioning. Under basic physiological conditions, dopaminergic neurons support normal functioning, both in motor and cognitive aspects¹². Processes associated with the loss of these neurons have been shown to impair cognitive functions¹². PD is a disease with progressive loss of dopaminergic neurons. These pathogenetic features can sufficiently explain the association found between the presence of cognitive deficit, the age of the patients and the age at the onset of complaints, but do not answer the question why some patients with early PD do not demonstrate cognitive dysfunction at diagnosis. Further studies are needed to reveal other factors influencing the cognitive functioning of patients with a view to precise and timely determination of risk and prevention strategy.

Conclusion

The presented study shows a significant incidence of cognitive impairment in patients with early PD. Patients' age and age at disease onset are factors associated with an increased risk of cognitive decline.

References

1. Aarsland D, Batzu L, Halliday G.M. et al. Parkinson disease-associated cognitive impairment. *Nat Rev Dis Primers* 7, 47 (2021).
2. Leroi I, McDonald K, Pantula H. et al. Cognitive impairment in Parkinson disease: impact on quality of life, disability, and caregiver burden. *J. Geriatr. Psychiatry Neurol.* 2012: 208–214.
3. Goldman JG, Sieg E. Cognitive impairment and dementia in Parkinson disease. *Clin Geriatr Med.* 2020; 36(2):365–77.
4. Chandler J. et al. Characteristics of Parkinson's disease in patients with and without cognitive impairment. (2021).
5. Goldman JG, Litvan I. Mild cognitive impairment in Parkinson's disease. *Minerva Med.* 2011 Dec; 102(6): 441-59.
6. Nagy A, Hardaker E, Bestwick JP, et al. Cognition in the PREDICT-PD cohort. 2017.
7. Petrova M, Raycheva M, Traykov L. Cognitive Profile of the Earliest Stage of Dementia in Parkinson's Disease. *American Journal of Alzheimer's Disease & Other Dementias®.* 2012; 27(8): 614-619.
8. Hemphill L, Valenzuela Y, Luna K, et al. Synergistic associations of depressive symptoms and aging on cognitive decline in early Parkinson's disease, *Clinical Parkinsonism & Related Disorders*, Volume 8, 2023: 2590-1125.
9. Litvan I, Aarsland D, Adler C.H, Goldman, J.G., et.al. MDS task force on mild cognitive impairment in Parkinson's disease: Critical review of PD-MCI. *Mov. Disord.*, 2011;26: 1814-1824.
10. Jaramillo-Jimenez A, Bocanegra Y, Buriticá O, et al. Subjective Cognitive and Communicative Complaints and Health-Related Quality of Life in Parkinson's Disease with and without Mild Cognitive Impairment, *Revista Colombiana de Psiquiatría (English ed.)*, Volume 52, Issue 4, 2023: 305-313.
11. Wan H, Wu D, Gao D, et. al. Disturbance of functional brain networks and cognitive decline in Parkinson's disease: Severe cerebral small vessel disease aggravates this relationship, *Parkinsonism & Related Disorders*, Volume 110, 2023: 105-386.
12. Irvani M, Shoaib M. Executive dysfunction and cognitive decline, a non-motor symptom of Parkinson's disease captured in animal models, *International Review of Neurobiology*, Academic Press, Volume 174, 2024: 231-255.

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Adverse effects of Libtayo (Cemiplimab) in the endocrine clinical practice – two cases of autoimmune hypophysitis during treatment for squamous cell carcinoma

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Introduction: Libtayo (cemiplimab) is an Immune checkpoint inhibitor (ICI) used to treat squamous cell carcinoma and non-small cell lung cancer (NSCLC). ICIs have resulted in a variety of side effects that involve the endocrine system, most commonly thyroiditis, hypophysitis, Type 1 diabetes, adrenalitis, and central diabetes insipidus. We present two clinical cases – 65-year-old patient with squamous cell carcinoma, treated with cemiplimab, who presented to the clinic with destructive thyrotoxicosis in the background of autoimmune thyroid disease and subsequent development of secondary hypocorticism. The other patient was started on Libtayo in relation to accompanying squamous cell carcinoma and carcinoma of the parotid gland. On admission, we registered primary hypothyroidism and central hypocorticism as a consequence of possible autoimmune hypophysitis that was suspected after performing MRI of the brain. **Discussion:** Autoimmune hypophysitis is considered a rare condition, some reports estimate an incidence of 1 in 9 million individuals. ICI-associated hypophysitis and primary adrenal insufficiency lead to a permanent loss of adrenal function that is potentially lethal. Clinical manifestations of hypocorticism, such as fatigue, weakness, poor oral intake, low blood pressure, nausea and vomiting overlap with symptoms of malignancy and may remain unrecognized which delays timely treatment. **Conclusion:** We present clinical cases of two patients with secondary hypocorticism caused by Cemiplimab in order to emphasize the importance of routine screening for ICI-hypophysitis as it has the potential to prevent significant morbidity and mortality.

Keywords: Libtayo, Immune checkpoint inhibitor, Secondary hypocorticism, Autoimmune hypophysitis

Introduction

Since the introduction of immune checkpoint inhibitors (ICIs) to the therapeutic arsenal of oncology, their indications continue to expand and they have become the standard of care for a number of malignancies due to their efficacy based on an anti-tumor immune response and subsequent impact on overall survival (1–7). One of the most commonly used ICIs are programmed death 1 (PD-1) inhibitors. PD-L1, the natural ligand of PD-1 in tumor cells and tumorinfiltrating macrophages, binds to PD-1 to induce and maintain immune tolerance. Activation of the PD-1/PD-L1 pathway inhibits T cell activation, eventually resulting in the escape of tumor cells from immune surveillance. Libtayo (Cemiplimab) is an Immune checkpoint inhibitor (ICI) used to treat skin cancer and non-small cell lung cancer. ICIs have resulted in a variety of side effects that involve the endocrine system, most commonly thyroiditis, hypophysitis, diabetes, adrenalitis, and central diabetes insipidus (7). Immune-related endocrine events (irEEs) are

considered one of the most common irAEs, accounting for 8.1% of all cases. The thyroid is the most commonly affected endocrine organ, followed by the pituitary. Type 1 diabetes mellitus (T1DM) and primary adrenal insufficiency were reported less frequently. Hypothyroidism usually develops from destructive thyroiditis. The majority of ICI-induced thyrotoxicosis is also caused by thyroiditis (7). Hypophysitis is defined as inflammation of the pituitary gland and infundibulum, commonly due to infectious, infiltrative, neoplastic, or autoimmune processes (7). Autoimmune hypophysitis is considered a rare condition, some reports estimate an incidence of 1 in 9 million individuals. ICI-associated hypophysitis and primary adrenal insufficiency lead to a permanent loss of adrenal function that is potentially lethal. The median time to presentation in patients treated with anti-PD-1/L1 therapy is 26 weeks. Adrenocorticotrophic hormone and thyroid-stimulating hormone deficiencies are very frequent in the early stages of hypophysitis and must be identified

immediately. Patients with adrenal insufficiency may experience fatigue, dizziness, nausea, vomiting and weight loss. We present two clinical cases – 65 year old patient with squamous cell carcinoma, treated with cemiplimab, who presented to the clinic with destructive thyrotoxicosis in the background of autoimmune thyroid disease and subsequent development of secondary hypocorticism. The other patient was started on Libtayo in relation to accompanying squamous cell carcinoma and carcinoma of the parotid gland. On admission, we registered primary hypothyroidism and central hypocorticism as a consequence of possible autoimmune hypophysitis that was suspected after performing MRI of the brain.

Clinical cases

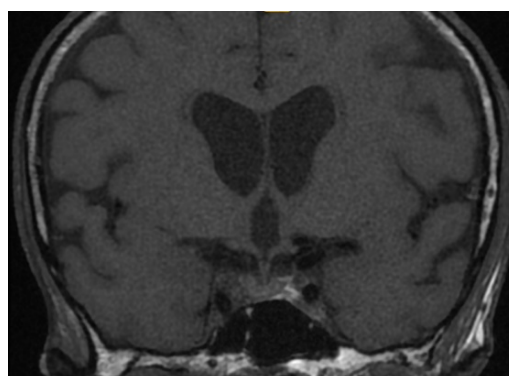
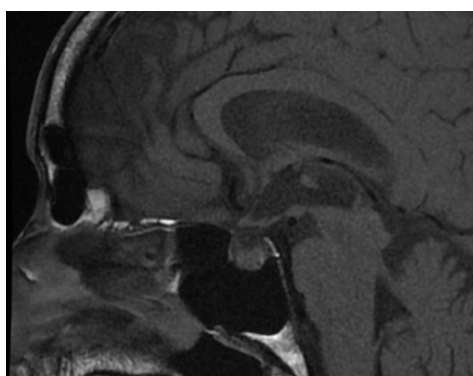
Patient 1: A 65-year-old patient with squamous cell carcinoma, treated with cemiplimab, presented to the clinic with destructive thyrotoxicosis in the background of autoimmune thyroid disease. During the follow-up, the patient entered a hypothyroid phase, necessitating replacement therapy with levothyroxine with a gradual dose increase to 100 µg/day. For the accompanying Type 2 diabetes he was on oral therapy with sulfonylurea, dipeptidyl peptidase-4 inhibitor and metformin, combined with temporary administration of intermediate insulin in connection to the worsening of his glycemic control at the background of the accompanying thyrotoxicosis. During a subsequent hospitalization the patient presented with central

hypocorticism, a tendency for hypoglycemia, which led to the need for discontinuation of antidiabetic therapy.

Patient 2: A 64-year-old man presented with complaints of weight loss, general fatigue. In 2017 he underwent a surgical intervention for nasal squamous cell carcinoma, reoperation due to recurrence of the process in 2019, as well as radiotherapy. In 2021 subtotaloadenectomy totalis sinistra was performed because of the development of moderately differentiated squamous cell carcinoma. In 2022 he was diagnosed with a recurrence and underwent radio-, chemo- and immunotherapy with Libtayo. He presented to the clinic with a constellation of primary hypothyroidism and negative thyroid antibodies; central hypocorticism – low morning serum cortisol and relatively low inappropriate ACTH values. MRI of the hypothalamic-pituitary region was performed and it showed an inhomogeneity of the pituitary parenchyma, after administration of contrast material, with a microadenoma (2 mm). As a differential diagnosis we considered the presence of lymphocytic hypophysitis as a cause of the adrenal insufficiency.

Table 1. Hormonal tests on admission

Parameter	Patient 1	Patient 2
TSH (0.34–5.6 mU/L)	0.093	17.431
FT3 (3.8–6 pmol/L)	-	3.02
FT4 (7.86–14.41 pmol/L)	37.02	9.64
ACTH (5–47 pg/ml)	16.8	14
Cortisol – morning (154–624 nmol/l)	97.97	49.94



MRI of the hypothalamic-pituitary region T1 of Patient 2

Both patients were started on glucocorticoid therapy and levothyroxine, which significantly improved clinical symptoms.

Discussion

Clinical manifestations of hypocorticism, such as fatigue, weakness, poor oral intake, low blood pressure, nausea and vomiting overlap with symptoms of malignancy and may remain unrecognized which delays timely treatment. All patients treated with ICIs should have their thyroid function assessed every 4–8 weeks or more frequently if clinically indicated (7).

Conclusion

We present clinical cases of two patients with central hypocorticism caused by Cemiplimab in order to emphasize the importance of routine screening for ICI-hypophysitis and adrenal insufficiency as it has the potential to prevent significant morbidity and mortality.

References

1. Albarran-Artahona V, Laguna JC, Gorria T, Torres-Jimenez J, Pascal M, Mezquita L, et al. Immune-related uncommon adverse events in patients with cancer treated with immunotherapy. *Diagnostics (Basel)* (2022) 12(9):1–31. doi: 10.3390/diagnostics12092091
2. Paz-Ares L, Ciuleanu TE, Cobo M, Schenker M, Zurawski B, Menezes J, et al. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. *Lancet Oncol* (2021) 22(2): 198–211. doi: 10.1016/S1470-2045(20)30641-0
3. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* (2017) 377(14):1345–56. doi: 10.1056/NEJMoa1709684
4. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-Small-Cell lung cancer. *N Engl J Med* (2018) 378(22):2078–92. doi: 10.1056/NEJMoa1801005
5. Garon EB, Hellmann MD, Rizvi NA, Carcereny E, Leighl NB, Ahn MJ, et al. Five-year overall survival for patients with advanced Non-Small-cell lung cancer treated with pembrolizumab: results from the phase I KEYNOTE-001 study. *J Clin Oncol* (2019) 37(28):2518–27. doi: 10.1200/JCO.19.00934
6. Albiges L, Tannir NM, Buratto M, McDermott D, Plimack ER, Barthé lémy P, et al. Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 4-year follow-up of the phase III CheckMate 214 trial. *ESMO Open* (2020) 5(6):e001079. doi: 10.1136/esmoopen-2020-001079
7. Cardona Z, Sosman JA, Chandra S, Huang W. Endocrine side effects of immune checkpoint inhibitors. *Front Endocrinol (Lausanne)*. 2023 May 11;14: 1157805. doi: 10.3389/fendo.2023.1157805. PMID: 37251665; PMCID: PMC10210589.
8. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* (2017) 545(7655):495–9. doi: 10.1038/nature22396
9. Mattox AK, Lee J, Westra WH, Pierce RH, Ghossein R, Faquin WC, et al. PD-1 expression in head and neck squamous cell carcinomas derives primarily from functionally anergic CD4(+) TILs in the presence of PD-L1(+) TAMs. *Cancer Res* (2017) 77(22):6365–74. doi: 10.1158/0008-5472.CAN-16-3453
10. Jiang Y, Chen M, Nie H, Yuan Y. PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations. *Hum Vaccin Immunother* (2019) 15 (5):1111–22. doi: 10.1080/21645515.2019.1571892
11. Lee PY, Oen KQX, Lim GRS, Hartono JL, Muthiah M, Huang DQ, et al. Neutrophil-to-Lymphocyte ratio predicts development of immune-related adverse events and outcomes from immune checkpoint blockade: a case-control study. *Cancers (Basel)* (2021) 13(6):1–14. doi: 10.3390/cancers13061308
12. Tan MH, Iyengar R, Mizokami-Stout K, Yentz S, MacEachern MP, Shen LY, et al. Spectrum of immune checkpoint inhibitors-induced endocrinopathies in cancer patients: a scoping review of case reports. *Clin Diabetes Endocrinol* (2019) 5:1. doi: 10.1186/s40842-018-0073-4
13. Wright JJ, Powers AC, Johnson DB. Endocrine toxicities of immune checkpoint inhibitors. *Nat Rev Endocrinol* (2021) 17(7):389–99. doi: 10.1038/s41574-021-00484-3
14. Villa NM, Farahmand A, Du L, Yeh MW, Smooke-Praw S, Ribas A, et al. Endocrinopathies with use of cancer immunotherapies. *Clin Endocrinol (Oxf)* (2018) 88 (2):327–32. doi: 10.1111/cen.13483
15. Chalan P, Di Dalmazi G, Pani F, De Remigis A, Corsello A, Caturegli P, et al. Thyroid dysfunctions secondary to cancer immunotherapy. *J Endocrinol Invest* (2018) 41(6):625–38. doi: 10.1007/s40618-017-0778-8

Nonbacterial thrombotic endocarditis – the path for the diagnosis

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Introduction: The nonbacterial thrombotic endocarditis is a rare disease often associated with hypercoagulable states such as systemic lupus erythematosus, antiphospholipid syndrome or advanced malignancy like adenocarcinoma. Clinical manifestation is frequently associated with an embolic incident. **Aim:** To describe a clinical case of nonbacterial endocarditis as a result of undiagnosed antiphospholipid syndrome and high suspicion for systemic lupus. **Materials and methods:** We present a case of 55 years old woman, who was admitted to the cardiology clinic due to persistent fevers up to 39 degrees for two months, arthralgias and myalgias. As a comorbidity, reported Crohn’s disease, for which remission was achieved on Mesalazine therapy. **Results and discussion:** Transthoracic and transesophageal echocardiography showed vegetation of the aortic valve, without any presence of valve dysfunction. In the presence of triple-negative blood cultures, persistence of fevers, and the presence of histologically proven autoimmune disease, further investigations were performed, which proved antiphospholipid syndrome and high clinical suspicion for systemic lupus. After consultation with a rheumatologist and administration of Methylprednisolone, the patient remained permanently afebrile. The patient was referred to a rheumatology clinic for further treatment. In patients with “suspected infective endocarditis” with formed vegetation and negative blood cultures, nonbacterial thrombotic endocarditis should be considered, due to a difference in therapeutic behavior. Anticoagulant therapy is the first line of treatment for nonbacterial endocarditis, because of the increased risk of systemic embolism, rather than antibacterial therapy. **Conclusion:** Treatment of the underlying disease in nonbacterial thrombotic endocarditis is key to the patient’s short- and long-term prognosis. **Keywords:** Nonbacterial thrombotic endocarditis (NBTE); Libman-Sacks endocarditis (LSE); Systemic lupus erythematosus (SLE); Antiphospholipid syndrome (APS)

Introduction

Lazare Riviere described the first case of infectious endocarditis (IE) in 1646, and Zeigler in 1888 the first case of nonbacterial. In 1936, the link between hypercoagulability and sterile vegetations was proven. Nonbacterial thrombotic endocarditis (NBTE) is characterized by the appearance of vegetations of fibrin and platelets in the absence of an infectious agent and represents a potential source of life-threatening thromboembolism. The disease is mainly associated with a hypercoagulable state – neoplasia, antiphospholipid syndrome (APS), systemic lupus erythematosus (SLE), and chronic inflammatory diseases such as tuberculosis.

Clinical case

A 55-year-old female patient turns to the cardiology clinic at St. George’s LLC, Plovdiv, for persistent fevers up to 38 degrees for

two months. As comorbidity, she reported Crohn’s disease, which was proven histologically in 2022. After starting therapy with Mesalazine, complete remission is achieved. A month before entering the clinic, she was treated for reactive arthritis, which manifested itself with pain in the small joints of the upper limbs, cervical thoracic region and morning stiffness for more than 30 minutes. Two weeks before the visit to the clinic, a panoramic radiography was taken, which visualized a granuloma on a second molar. Therapy with Amoxicillin/Clavulanic acid was started and the tooth was extracted.

The initial evaluation reveals subfebrile up to 37.2°C. She presents with a rhythmic heart activity with a frequency of 85 beats per minute, clear tones, without any murmurs. Her blood pressure was 90/60 mmHg, which are usual for her. The performed transthoracic echocardiography (TTE) revealed a

soft structure along the non-coronary aortic leaflet, 0.8×0.8 cm, with mild aortic regurgitation. The transesophageal echocardiography (TEE), confirmed the findings.

The patient was diagnosed with “suspected IE” according to the modified *Duke* criteria with the presence of 1 major and 1 minor criteria. 3 pairs of blood cultures were taken which were negative. From the laboratory tests, an insignificant movement of the inflammatory markers was established, without evidence of leukocytosis. Empiric antibiotic therapy with *Vancomycin* and *Cefepime* was started, and on day 10 the cephalosporin was replaced with *Amikacin*. During the serial echocardiographic and laboratory follow-up of the patient, no dynamics were found in the markers of inflammation or progression of valvular regurgitation, with daily persistence of febrile peaks that were successfully stopped with *Paracetamol*. A Full Body Scan was performed, from which no pathological finding was established, as well as a *Mantoux* test for tuberculosis – anergic /5 mm/ *Candida albicans* was isolated from coproculture, for which treatment with *Nystatin* was started.

The lack of effect from the administration of antibiotics, the persistence of fevers, the absence of valvular destruction and the non-significantly elevated markers of inflammation made us doubt the diagnosis of infective endocarditis. According to the latest *ESC* recommendations for the management of endocarditis, an immunological panel was taken, from which *APS* and *SLE* were proven, with marked clinical activity and 14 points according to the updated classification of the *American College of Rheumatology* and the *European Alliance of Associations for Rheumatology* from 2018. After starting low-dose corticosteroid therapy: *Methylprednisolone* 20 mg, fevers disappeared, the general condition of the patient

improved and inflammatory markers normalized.

Discussion

Nonbacterial thrombotic endocarditis is a rare disease that should be considered in the presence of suspected IE, negative blood cultures, non-significantly elevated markers of inflammation, and absence of valvular destruction.

Most commonly, the disease affects the fourth-eighth decade of life, regardless of gender. *NBTE* is often asymptomatic and remains unrecognized during lifetime. *Bussani et al., 2019, Yoo et al., 2020, Liu & Frishman, 2016* reported involvement of left-sided heart valves with a slight predominance of mitral over aortic. *Lopez et al., 1987* in the *American Heart Journal* presented one of the first detailed analyzes of the disease. The pathogenesis goes through various theories, including Vitamin C deficiency, chronic hypoxia and “hemodynamic trauma” with development of endothelial dysfunction, until in 1956 *MacDonald and Robbins* proved the relationship with hypercoagulability, and *Trousseau* was the first to associate the disease with neoplasia. The main predisposing factors are: autoimmune process, *APS* (primary or secondary with *SLE*), chronic inflammatory disease such as tuberculosis and adenocarcinomas.

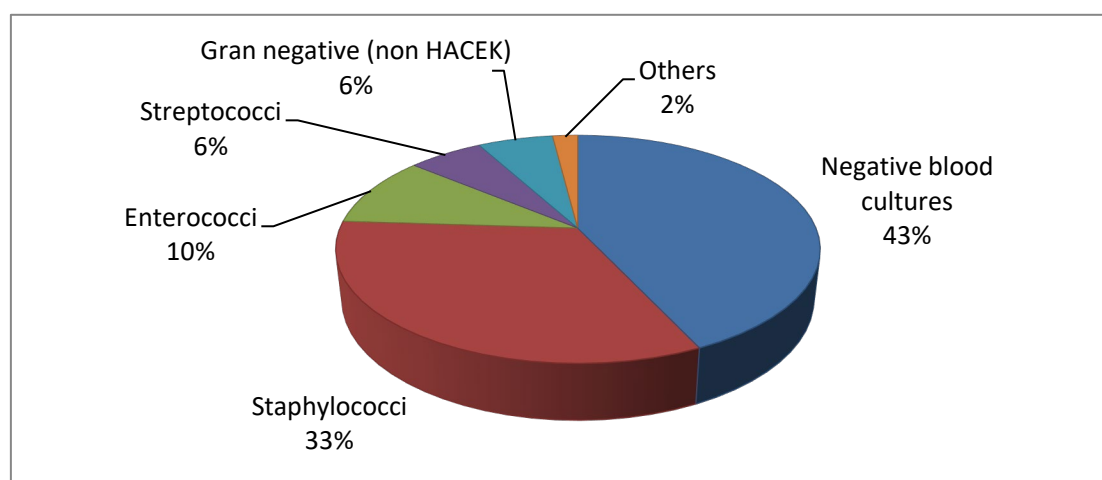
NBTE has no pathognomonic signs and only high clinical suspicion helps to make the diagnosis in time. *McKay and Wahler* 1954 were among the first to propose a clinical triad to aid the diagnosis: 1. a predisposing condition for the development of *NBTE*, 2. the presence of a heart murmur, 3. evidence of disseminated systemic embolism. This triad, accompanied by evidence of venous thrombosis, a laboratory constellation of *DIC* syndrome, and a series of negative blood cultures support the diagnosis of *NBTE*. Numerous analyzes since 1954 show

the high rate of embolism, average of 42% (14.1 to 90.9%).

The current approach to diagnosis is based on the latest *ESC* guideline for endocarditis *Delgado et al., 2023* and is based on exclusion. First of all, infective endocarditis caused by typical microorganisms, mycotic endocarditis and infective endocarditis caused by atypical agents such as *C. burnetti*, *Bartonella spp.*, *Asperigilus spp.*, *L. pneumophila*, *Brucella spp.*, *M pneumonia* must be excluded. Serological tests and *PCR* help to get faster results. IE with negative blood cultures is increasing as a percentage, mainly because antibiotic therapy is started before meeting with a cardiologist. Our study *Dobreva-Yatseva et al., 2023* shows that infective endocarditis with negative blood cultures represents 43% of cases treated in the clinic of 270 patients for the period 2005–2021. When an infectious agent is excluded, antinuclear antibodies, antiphospholipid antibodies and Anti-pork antibodies are tested. Standard laboratory tests are further helpful: normal leukocyte count, non-significantly elevated *CRP*, procalcitonin and fibrinogen, and the presence of antiphospholipid antibodies support the diagnosis of *NBTE*. *TEE* has higher sensitivity

and specificity than *TTE*. The usual findings are small vegetations, less than 1 cm, and the absence of valvular destruction. Multimodality imaging helps to detect neoplasias.

Unlike IE, the key to *NBTE* treatment is anticoagulation because of the high risk of systemic embolism. The predilection site for embolization is the central nervous system with priority to the brain and the clinical presentation of stroke and/or transient ischemic attack. Characteristic of the involvement of the brain is multi-infarct encephalopathy. Neurological complications are more common in *NBTE* than in IE. Over the years and with experience, the anticoagulation strategy changes. The benefits of unfractionated herarin are indisputable, vitamin K antagonists from non-recommended drugs in the past are currently the gold standard in *NBTE* in combination with *APS* with target *INR* levels of 2–3. The treatment strategy also includes the administration of corticosteroids to control the underlying disease. *Liu & Frishman, 2016* recommend the use of lower doses of corticosteroids due to increased valvular fibrosis and progression of dysfunction. As yet, therapy for *NBTE* is not based on randomized clinical trials.



Distribution of patients with infective endocarditis according to blood culture isolate, Dobreva-Yatseva et al., 2023

Labs on administration

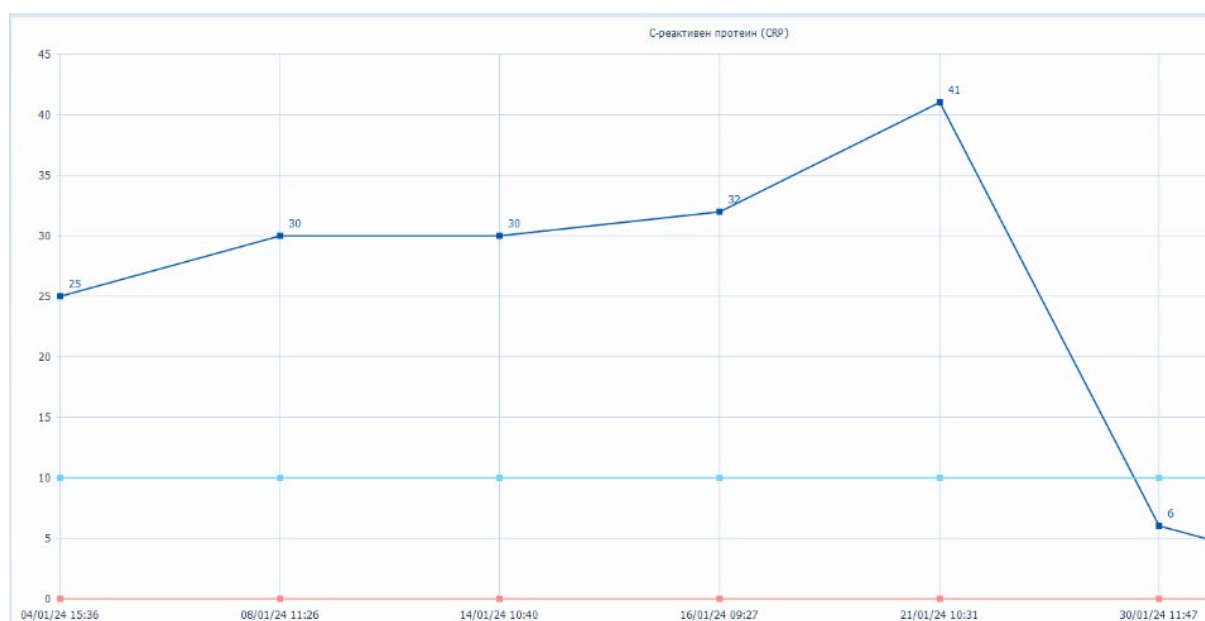
Complete blood count		Range
HBG	133	120–160 g/L
RBC	4.6	3.9–5.3x10 ¹²
HCT	0.407	0.36–0.47 L/L
WBC	5.7	3.5–10.5x10 ⁹
PLT	224	140–400x10 ⁹
Differential blood count		
Sg	4.37	2.2–6.6x10 ⁹
EOS	0.01	0–0.5x10 ⁹
BASO	0.01	0–0.5x10 ⁹
MONO	0.15	0–0.5x10 ⁹
LYMPH	1.23	0–0.5x10 ⁹
ESR (Westergen)	50	2–30 mm/h
Clinical chemistry		
CRP	25	0–10 mg/L
CREA	71	44–96 μmol/L
UR AC	206	149–363 μmol/L
UREA	3.6	2.6–7.2 mmol/L
GLUC	3.4	2.8–6.1 mmol/L
AST	54	0–35 U/L
ALT	55	0–35 U/L
Coagulation		
PT	120	70–120%
INR	0.87	
APTT	20.8	24–35 sec
FBG	5.55	2.0–4.5 g/L

Immunology panel

Immunology tests		Range
total ANA	1:280 /4+/-	
LA	81%	70–100%
anti-AP-Ab IgG	33	<10 U/ml
ACA IgG	50.7	<10
C ₃	1.32	0.90–1.80 g/L
C ₄	0.28	0.10–0.40 g/L
anti-dsDNA Ig	negative	
anti-Histone Ab	negative	
U-CSF PROT	1.01	0–0.15 g/L
anti-Smith Ab	negative	



TEE: vegetation on noncoronary cusp



Dynamic of CRP levels during treatment

Conclusion

NBTE is a rare disease that should be actively considered in the presence of vegetation, negative blood cultures, non-significantly elevated markers of inflammation and lack of effect of antimicrobial therapy. A major focus in therapeutic behavior is anticoagulation, and prognosis is related to the underlying disease.

References

1. Delgado, V., Marsan, N. A., De Waha, S., Bonaros, N., Brida, M., Burri, H., Caselli, S., Doenst, T., Éderhy, S., Erba, P. A., Foldager, D., Fosbøl, E. L., Kováč, J., Mestres, C. A., Miller, O., Miró, J. M., Pazderník, M., Pizzi, M., Quintana, E., . . . Zeppenfeld, K. (2023). 2023 ESC Guidelines for the management of endocarditis. *European Heart Journal*, 44(39), 3948–4042. <https://doi.org/10.1093/eurheartj/ehad193>
2. Bussani, R., De-Giorgio, F., Pesel, G., Zandonà, L., Sinagra, G., Grassi, S., Baldi, A., Abbate, A., & Silvestri, F. (2019). Overview and comparison of infectious endocarditis and non-infectious endocarditis: A review of 814 autopsic cases. *In Vivo/in Vivo*, 33(5), 1565–1572. <https://doi.org/10.21873/invivo.11638>
3. Yoo, B. S., Lee, S. W., Song, J. J., Park, Y. B., & Jung, S. M. (2020). Clinical characteristics and long-term outcomes of Libman–Sacks endocarditis in patients with systemic lupus erythematosus. *Lupus*, 29(9), 1115–1120. <https://doi.org/10.1177/0961203320930097>
4. Liu, J., & Frishman, W. H. (2016). Nonbacterial thrombotic endocarditis. *Cardiology in Review*, 24(5), 244–247. <https://doi.org/10.1097/crd.000000000000106>
5. Lopez, J., Ross, R. S., Fishbein, M. C., & Siegel, R. J. (1987). Nonbacterial thrombotic endocarditis: A review. *American Heart Journal/the American Heart Journal*, 113(3), 773–784. [https://doi.org/10.1016/0002-8703\(87\)90719-8](https://doi.org/10.1016/0002-8703(87)90719-8)
6. Dobрева-Yatseva, B., Nikolov, F., Raycheva, R., Manolov, I., Nikolov, P., Ivanova, N., Iovanovska, D., Kuchmov, I., & Tokmakova, M. (2023). Characteristics of infective endocarditis according to the mode of acquisition – a single-center, retrospective analysis. *B"lgarska Kardiologiâ/B"lgarska Kardiologiâ*, 29(2), 24–35. <https://doi.org/10.3897/bgcardio.29.e108621>

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Epigenetic and morphological aspects of healthy aging with functional foods enriched in gamma-aminobutyric acid and polyphenolic compounds

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Introduction: The aging is a relatively new problem in human history. **Aim:** The main objective of the project is to study the effect of functional foods and beverages enriched in biologically active compounds in *in vitro* and *in vivo* models of aging by the epigenetic modulation of the α -Klotho gene. **Materials and methods:** This includes the development and characterization of functional foods and beverages enriched with gamma-aminobutyric acid and polyphenolic compounds, investigating the effect of the developed functional foods and beverages on delaying the aging process in *in vitro* models of aging and investigating the subtle molecular pathways of influencing some of the hallmarks of cellular aging, including the epigenetic modulation of the α -Klotho gene, studying the effect of functional foods and beverages on *in vivo* rat models with healthy experimental animals undergoing spontaneous aging by examining cognitive, sensory, locomotor and other physiological functions after supplementation, as well as an in depth analysis of the morphological, molecular and epigenetic mechanisms of the antiaging effect in the kidney and the brain. Due to the multidisciplinary nature of this project, necessitating investigations spanning phytochemical, microbiological, epigenetic, biochemical, and medical domains, its execution will be undertaken collaboratively by three organizations. The significance and timeliness of this endeavor stem from the profound societal and economic implications associated with promoting healthy aging, a paramount concern of the 21st century. Its novelty and originality are underscored by the limited existing literature exploring the impact of nutritional components on Klotho gene expression, highlighting the pioneering nature of this research initiative.

Keywords: aging, functional foods and beverages, gamma-aminobutyric acid, polyphenolic compounds, Klotho gene

Ageing is a relatively new problem in human history, the urgency of which is constantly increasing (1). A promising strategy for healthy ageing is the ability to influence the ageing process with functional foods. By definition, epigenetics represents the reversible heritable mechanisms that occur without any alteration of the underlying DNA sequence. Although the chromosomes in our genome carry the genetic information, the epigenome is responsible for the functional use and stability of that valuable information; that is, it connects the genotype with the phenotype (2). Among the various hallmarks of aging, epigenetic alterations have emerged as a particularly compelling focus for extending lifespan. Unlike many other

processes associated with aging, epigenetic modifications offer promising avenues for intervention through the modulation of relevant enzymes, presenting opportunities for inhibition or stimulation to potentially mitigate age-related effects. Thus, interventions targeting epigenetic information hold immense potential to extend life span and to counteract age-associated diseases, including cancer and neurodegenerative disorders (3). Recently, the interest of the scientific community in the field of biology of aging has turned to the definition of other more precise markers of aging, such as the α -Klotho gene. It was first discovered in rats in 1997 (4) and has been shown to have two major forms: α -Klotho (α -Klotho) and

β -Klotho (β -Klotho) (5), which encode transmembrane proteins with important roles in various cellular processes. α -Klotho is highly evolutionarily conserved across animal species, with more than 98% homology found between humans and mice (6). It occurs in two forms, one as a membrane protein and the other in a secreted form (s-Klotho) (7). Levels of α -Klotho have been found to decline with age, as well as due to and during various diseases. On the other hand, overexpression of α -Klotho has been shown to be associated with longer lifespan and reduced risk of a number of diseases (8).

The main objective of the project we present is to investigate the effect of functional foods and beverages enriched with biologically active compounds in *in vitro* and *in vivo* models of aging by epigenetic modulation of the α -Klotho gene. The significance and relevance of this topic arise from the recognition that healthy aging constitutes one of the foremost social and economic challenges of the 21st century. The novelty and originality of this study are underscored by the limited existing research in the global literature concerning the impact of dietary components on the expression of the Klotho gene.

In order to investigate the epigenetic effects of functional foods rich in gamma-aminobutyric acid and polyphenols, we plan to carry out the project in several stages.

Stage I: In the first step it is planned to develop the functional foods themselves enriched with gamma-aminobutyric acid and polyphenols in the laboratory for biologically active substances at the Institute of Organic Chemistry with the Centre of Phytochemistry at BAS, Plovdiv. These foods are 100% juice of Aronia, pure substance gamma amino butyric acid (GABA), combination of Aronia and GABA, functional food with high natural content of GABA obtained

from sprouted chickpea. We are planning to test these foods on cell cultures at the Institute of Molecular Biology at BAS Sofia in order to establish preliminary data on their effect and estimate the doses for the next stage of the experiment. More substances and concentrations will be tested with the cell cultures in order to track more easily epigenetic changes. The acquired data will be useful in elucidating the effects in rats.

Stage II: The study protocol entails supplementing naturally aged male Wistar rats, aged 24 months, with newly developed food formulations for a duration of 3 months. This intervention will be conducted at the Vivarium of MU-Plovdiv. The following experimental groups will be formed: 1. YC – 3 months old young controls, without supplementation; 2. OC – 24 months old controls, without supplementation; 3. A – 24 months old rats supplemented with Aronia juice at a dose of 10 ml per kg weight; 4. G + A – 24 month old rats supplemented with pure GABA combined with 100% Aronia juice at a dose of 10 ml/kg bw; 5. G – 24 month old rats supplemented with pure GABA at a dose of 10 ml/kg bw; 6. NG – 24 month old rats supplemented with a functional food with high natural GABA content derived from sprouted chickpea. During the 3-month supplementation period, weight control of the animals will be performed every 2 weeks to optimize the supplementation doses.

Stage III: The third stage of the experiment will involve performing functional studies of cognitive and motor abilities of the rats using the following test: 1. Evaluation of the locomotor activity (Activity cage test); 2. Active learning (Shuttle-box); 3. Passive learning (Step-through, Step-down tests); 4. New object recognition test; 5. Spatial memory test (Y-maze); 6. Anxiety test (elevated plus maze). The tests will be carried out in the Department of Pharmacology, Faculty of Pharmacology, MU-Plovdiv.

Stage IV: The last stage of the experiment involves taking material for three types of studies: 1. Biochemical studies of blood and tissue homogenates of markers of oxidative stress, inflammation, serum klotho fraction and lipid profile to be studied in the Department of Medical Biochemistry, Pharmacy faculty, MU-Plovdiv. 2. Morphological studies of klotho gene and aging markers – histological, histochemical, immunohistochemical and morphometric in brain and kidney, which will be performed in the Department of Anatomy, Histology and Embryology, MF, MU-Plovdiv. 3. Genetic studies of Klotho in tissue homogenates from brain and kidney, which will be performed at the Institute of Molecular Biology, BAS, Sofia.

The expected outcomes after supplementation are increased Klotho gene activity, decreased levels of inflammatory markers, increased antioxidant capacity of tissues and serum, and improved cognitive and locomotor functions in supplemented animals.

The reported data are related to the forthcoming implementation of the project KP-06-N71/4 of 05.12.2023 at FNI on “Epigenetic and morphological aspects of healthy ageing with functional foods enriched in gamma-aminobutyric acid and polyphenolic compounds”.

References

1. Beard, J.R., Officer, A., de Carvalho, I.A., Sadana, R., Pot, A.M., Michel, J.-P., Lloyd-Sherlock, P., Epping-Jordan, J.E., Peeters, G.M.E.E. (Geeske), Mahanani, W.R., Thiyagarajan, J.A., Chatterji, S., 2016. The World report on ageing and health: a policy framework for healthy ageing. *Lancet* 387, 2145–2154.
2. Muñoz-Najar, J. M. Sedivy, Epigenetic control of aging. *Antioxid. Redox Signal.* 14, 241–259 (2011)
3. R. J. O’Sullivan, J. Karlseder, The great unraveling: Chromatin as a modulator of the aging process. *Trends Biochem. Sci.* 37, 466–476 (2012).
4. Kuro-o, M. (2008). "Klotho as a regulator of oxidative stress and senescence." *Biol Chem* 389(3): 233-241.
5. Donate-Correa, J., B. Martín-Carro, J. B. Cananata-Andía, C. Mora-Fernández and J. F. Navarro-González (2023). "Klotho, Oxidative Stress, and Mitochondrial Damage in Kidney Disease." *Antioxidants (Basel)* 12(2).
6. Wang, Y. and Z. Sun (2009). "Current understanding of klotho." *Ageing Res Rev* 8(1): 43-51.
7. Xu, Y. and Z. Sun (2015). "Molecular basis of Klotho: from gene to function in aging." *Endocr Rev* 36(2): 174-193.
8. Sopjani, M., M. Rinnerthaler, J. Kruja and M. Dermaku-Sopjani (2015). "Intracellular signaling of the aging suppressor protein Klotho." *Curr Mol Med* 15(1): 27-37.

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Prolactinoma: a case study

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Prolactinoma is a tumor of the adenohypophysis, originating from the lactotrophic cells, which chronically secretes prolactin in abnormally high levels and causes gonadic dysfunction. Usually, the adenoma presents itself with galactorrhea, oligorrhea, amenorrhea and/or anovulation with shortened luteal phase, severe premenstrual syndrome, low libido, mild hirsutism, and seborrhea. This case report presents how even though the patient presents with regular menstrual cycle and normal sex hormone levels, the presence of ovulatory bleeding, which could appear to be a gynecological issue, might be the debut of an underlying endocrinological condition. We present a young woman, whose main symptom is ovulatory bleeding, which has gone from slight spotting to severe hemorrhage for 5–7 days a week over the years. Broad spectrum of hormonal assays was made such as prolactin, macroprolactin, pituitary-gonad and thyroid hormone levels. The results showed drastically elevated prolactin levels. A magnetic resonance imaging was done. A tumor was found with parameters of a microadenoma (8.9 mm transversal, 5.1 mm craniocaudal and 3.5 mm sagittal). In a differential diagnostic are discussed a Polycystic Ovary Syndrome, Idiopathic hyperprolactinemia, or Autoimmune hypothyroidism, due to the probability of Hashimoto antibodies creating complexes with prolactin, resulting in macroprolactin. In conclusion, a symptom of bleeding during ovulation can be related to an endocrine disturbance such as hyperprolactinemia, which is not a classical and common symptom.

Keywords: prolactinoma, ovulatory bleeding, prolactin

Introduction

Prolactinoma is the most common hormone-producing tumor of the adenohypophysis. It chronically secretes prolactin in abnormally high level, causing galactorrhea, oligorrhea, amenorrhea and/or anovulation, with shortened luteal phase, severe premenstrual syndrome, low libido, mild hirsutism and seborrhea. Epidemiologically, it affects mostly women age between the age of 20 and 40. In recent years the clinical manifestation has shown alterations, including atypical or lack of symptoms.^[1]

Case presentation

The patient is a 22-year-old woman, whose symptoms have started when she was 18 and progressed through the years. The patient described her episodes of bleeding during her ovulation as having the tendency to appear once in three months and then, in the course of a few years, progressing to each ovulation of a menstrual cycle. The duration varied month by month, with stress being the main factor. Her menstrual cycles have always been regular.

In 2020, when the episodes became monthly, a gynecologist consultation was performed. Broad spectrum of hormonal assays was made including assessment of prolactin, pituitary-gonad axis including androgens and pituitary-thyroid axis. The results showed significant elevated prolactin levels.

Over the course of the next three years, no significant progression of the symptoms was noted.

In 2023 she sought the help of an Endocrinologist, because of worsened symptoms of ovulatory bleeding. Her menstruations were still regular.

Examination and laboratory results

In 2020, the hormonal assays showed prolactin levels of 1116 $\mu\text{IU/L}$ (reference values: 101.76–487.60 $\mu\text{IU/L}$). The patient was consulted with an endocrinologist and additional hormonal tests were referred: Progesterone of 0.90 ng/ml in luteal phase (reference values: 1.83–23.9 ng/ml) and prolactin of 1134 $\mu\text{IU/L}$. The patient had preserved menstrual cycle despite the progesterone levels.

In 2023 during an ultrasonographic examination, heteroechogenic lesions were found in her thyroid's right lobe. She denied family history of goiter or autoimmune conditions. After blood tests, the level of prolactin was estimated to be 1131 μ IU/l and test results for macroprolactin being monomeric form of 964.62 μ IU/L and % Recovery estimated as 73.21%. Thyroid stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), antithyroglobulin antibodies (TAT) and antimicrosomal antibodies (MAT) were normal.

Diagnostic imaging

On an MRI scan two lesions were found. One of them, after being applied a contrast matter, was described as having sizes of 5.1 mm craniocaudally, 8.9 mm transversally and 3.5 mm sagittal – a microadenoma.

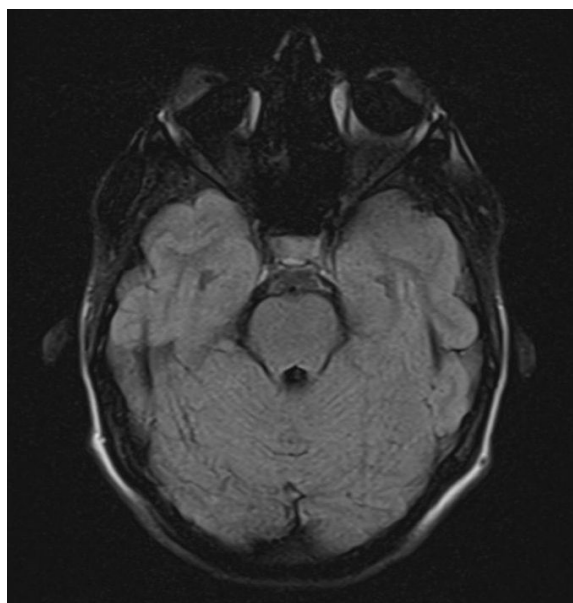


Figure 1. MRI scan of the pituitary gland

Treatment

The patient was prescribed Cabergoline (Dostinex) 0.5 mg, which she should take each week, half a tablet twice a week and was told to have new blood tests ran in three months to evaluate the doses of the treatment and if there should be any corrections.

Three months after starting the treatment, prolactin levels have been reduced to 13 μ IU/L and the dosage of Dostinex has been reduced by half. The patient shared about improvement of her bleeding and reducing the severity of her pre-menstrual syndrome.

Discussion

The patient presents with none of the traditional symptoms of a prolactinoma (amenorrhea, galactorrhea or oligo-/anovulation), but during the course of her treatment she shared about improvement of other non-specific symptoms (seborrhea and premenstrual symptoms). This clinical manifestation led to the idea of the patient having falsely elevated prolactin due to higher than normal macroprolactin. She denied being tested for it. Macroprolactin is a complex of prolactin and anti-prolactin antibodies, mainly IgG subtype. Sometimes those antibodies could bind with the receptors for prolactin and we could have elevated levels of the hormone and low biological activity.

The etiology behind macroprolactinemia is unclear. There is a theory of a defect in the glycosylation and phosphorylation of the prolactin molecule, which in turn makes it immunogenic. According to other authors, in some patients with autoimmune hypo- or hyperthyroidism, SLE and etc. there could be estimated high levels of prolactin and macroprolactin, but it is not seen in every patient with said conditions.^[2,3]

The theory was denied when the patient's test results showed lack of macroprolactin.

In a differential diagnostic plan was the possibility of the patient having a second underlying condition: Hashimoto's hypothyroiditis. Hypothyroidism debuts with low levels of fT3, fT4. The low peripheral hormones cause the hypothalamus to produce more thyroglobulin releasing hormone

(TRH) and subsequently more TSH from the pituitary gland. TRH stimulates the production of prolactin from the lactotrophic cells and this is the reason why patients with hypothyroidism could have secondary elevated prolactin. The findings from the ultrasonographic exam were not significant, but still the patient had her thyroid hormones tested. The results came back normal.^[4]

The diagnosis Polycystic Ovarian Syndrome was denied due to the patient not covering neither of the Rotterdam criteria: oligo-/anovulation, hyperandrogenism and polycystic ovaries, seen during ultrasonographic scanning.^[5]

Idiopathic hyperprolactinemia was excluded after the MRI findings.

Conclusion

This case represents how in recent years prolactinomas are underdiagnosed not due to lack of diagnostic methods but because of how they could mask their presence and

debut with atypical symptoms. This condition proved how little is known about prolactin and its influence on the human body. Furthermore, macroprolactin is still very unknown to the medical sphere, but very important when we diagnose our patients, because we can be sure to give them the right diagnosis and, therefore, treatment.

References

1. Prof. F. Nikolov and Co. "Internal diseases" p. 556-559
2. Richa Vaishya, Rahul Gupta, Sarika Arora "Macroprolactin; A Frequent Cause of Misdiagnosed Hyperprolactinemia in Clinical Practice"
3. Akira Shimatsu, Naoki Hattori "Macroprolactinemia: Diagnostic, Clinical, and Pathogenic Significance"
4. Prof. F. Nikolov and Co. "Internal diseases" p. 533
5. Jacob P. Christ, Marcelle I. Cedars "Current Guidelines for Diagnosing PCOS"

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Microbiological diagnosis and treatment strategy for invasive *Candida glabrata* infections

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Introduction: *Candida glabrata* is an opportunistic pathogen and the second or third most common causative agent of candidiasis depending on the site of infection. This pathogenic fungus is a therapeutic problem due to a constant decrease in antifungal susceptibility. Treatment for *C. glabrata* infections is challenging since the pathogen frequently develops resistance to several echinocandins and azoles, especially fluconazole. As a result, immunocompromised patients have a significantly higher mortality rate from *C. glabrata* infections. **Aim:** The aim of this study was to explore the available scientific literature for summarising the recommended approaches for microbiological diagnosis and therapy in patients with invasive *C. glabrata* infections. **Materials and methods:** A PubMed search on the relative scientific literature with keywords: *Candida glabrata*, invasive infections, and treatment was performed. Articles that investigated the treatment of invasive *C. glabrata* infection were included as well as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. **Results and discussions:** According to EUCAST and the available literature, the recommended antifungals for *C. glabrata* antimycogram are micafungin, anidulafungin, caspofungin, fluconazole, and amphotericin B. In recent years there has been an increase in resistance of *C. glabrata* to azoles and echinocandins. This poses a serious therapeutic problem as it eliminates treatment options for invasive *C. glabrata* infections in immunocompromised patients. **Conclusion:** To tackle the problem of increasing antimycotic resistance and mortality in invasive *C. glabrata* infections, it is necessary to discover novel therapeutic approaches and markers that distinguish invasive from superficial candidiasis.

Keywords: Fungal infection, invasive mycotic infection, antifungal treatment, candida

Introduction

Candida glabrata is an opportunistic pathogen and the second or third most common causative agent of candidiasis depending on the site of infection. Candidemia is the most prevalent manifestation of invasive candidiasis which significantly increases mortality (1). The gastrointestinal tract's commensal, *Nakaseomyces* (previously *Candida*) *glabrata*, is ranked as a high-priority pathogen in the first World Health Organisation fungal priority list (2, 3). This pathogenic fungus is a therapeutic problem due to a constant decrease in antifungal susceptibility. Treatment for *C. glabrata* infections is challenging since the pathogen frequently develops resistance to several echinocandins and azoles, especially fluconazole. As a result, immunocompromised patients have a significantly higher mortality rate from *C. glabrata* infections.

Aim

The aim of this study was to explore the available scientific literature and summarise the recommended approaches for microbiological diagnosis and therapy in patients with invasive *C. glabrata* infections.

Materials and methods

A PubMed search on the relative available literature with keywords: *Candida glabrata*, invasive infections, and treatment was performed. Articles that investigated the treatment of invasive *C. glabrata* infection were included as well as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Vol.10.0. In addition, in some articles antimycotic resistance was determined using Clinical and Laboratory Standards Institute (CLSI) recommendations. The results from the literature search

were compared with several blood culture isolates from the Laboratory of Microbiology at University Hospital “St. George”, Plovdiv. The susceptibility of those *C. glabrata* strains in our laboratory was assessed using the Epsilometer test (E-test), Liofilchem®, Italy to determine the minimum inhibitory concentration (MIC) of Andulafungin and Itraconazole. Additionally, the MIC of micafungin and amphotericin B was determined using the VITEC 2 Compact, BioMerieux, France equipment method.

Results and discussions

Epidemiology

Approximately 90–95% of the *Candida* isolates in the 2008–2009 SENTRY study, which included isolates from 79 medical centers, belonged to five species: *Candida*

albicans, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* (4). However, there is a considerable variation in the prevalence of invasive candidiasis caused by *Candida* species other than *Candida albicans* among hospitals and patients, as well as in the distribution of *C. albicans* and *non-C. albicans Candida* species producing candidaemia. The *non-albicans Candida* (NAC) has an intriguing patient-specificity and specific geographic distribution. Moreover, there are some important geographical differences in the distribution of those *Candida species* different from *C. albicans* causing invasive candidiasis. *Candida glabrata* is the predominant causative agent of invasive candidiasis in North America and Northern-Central Europe relative to other *Candida species* (Fig. 1) (1).



Figure 1. Distribution of most frequently species of *Candida* other than *Candida albicans* (According to Quindos G., 2014)

Diagnosis

Diagnosis poses many challenges, and conventional culture techniques are frequently supplemented by non-culture-based assays such as PCR and gene sequencing. There is no substantial difference between diagnostic methods of non-invasive and invasive *Candida glabrata* infections (4).

Following identification of the pathogen it is necessary to perform antimicrobial susceptibility testing (AST). It is established that empirical treatment without AST leads to an increase in antifungal resistance in *C. glabrata* as well as in all *Candida spp.*

Resistance

One significant challenge in managing *Candida glabrata* infections is its increasing resistance to antifungal agents. This resistance can emerge due to several factors, including prolonged or inappropriate use of antifungal medications, the ability of the yeast to adapt rapidly to its environment, and genetic mutations that confer resistance.

The antifungal treatment is limited to 3 extant drug classes with azoles, echinocandins, polyenes and flucytosine as the main classes.

New surveillance studies have shown that triazole resistance has grown among *C. glabrata* isolates to the point where these drugs cannot be relied on for therapy without first being tested for susceptibility. In the US, Pfaller et al. reported fluconazole resistance rates in *C. glabrata* isolates of the order of 11–13% (5, 6). Testing of *C. glabrata* isolates from blood cultures reveals a relatively high proportion of fluconazole-resistant *C. glabrata*, including 16–22% (7). In another study, Oxman et al. found that 51% of

C. glabrata tested were resistant to fluconazole (8). Therefore, fluconazole therapy should not be initiated in *C. glabrata* infections without susceptibility testing of each strain. It turns out that the study by Hesdachi et al. in 2023 demonstrated preserved in vitro activity to itraconazole (7). Voriconazole has shown to be effective in both mucosal and invasive candidiasis. Its clinical use is primarily for step-down oral therapy in patients with infection due to fluconazole-resistant, voriconazole-susceptible *C. glabrata* (9). All azoles have lower activity against *C. glabrata* than against other *Candida species*, hence treatment with this group must be administered after antimicrobial susceptibility testing.

The fluconazole resistance was variable and exhibited growth from 13% in 1992–1993 16–22% in 2012–2022, as visually demonstrated when overlaid on a time curve (Fig. 2).

A comparable pattern of resistance started to emerge in a higher percentage of *C. glabrata* isolates against echinocandins (Fig. 3).

Back in 2013, Alexander et al. documented the widespread emergence over time of resistance to both fluconazole and echinocandins in clinical isolates of *C. glabrata* from a single tertiary care center (Fig. 4). Prior exposure to echinocandins and the existence of FKS genes mutations have been identified as predictors of resistance to these drugs. Both factors are related to clinical treatment failure in these infections (10).

There is no substantial increase in amphotericin B resistance reported from 2012–2022 by CLSI (0%) and EUCAST (1.2%) (7).

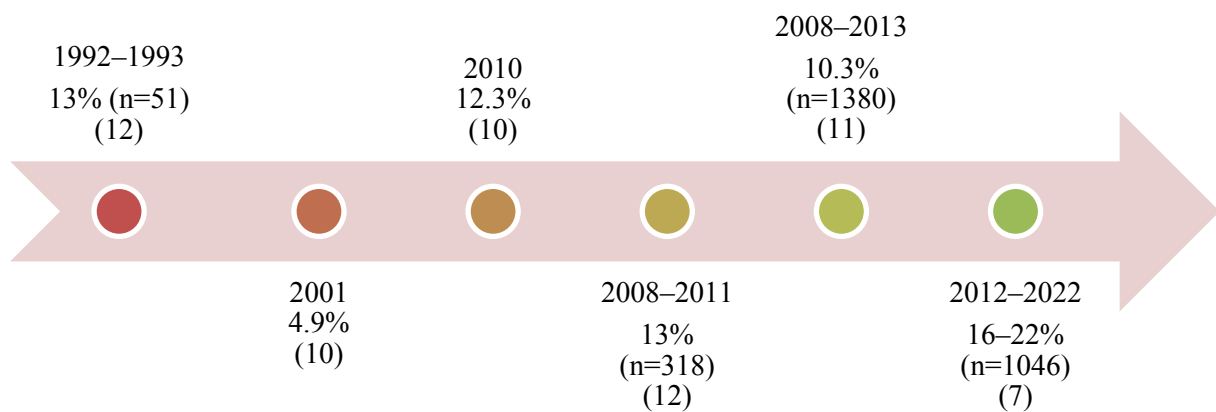


Figure 2. Distribution of Fluconazole resistance to *C. glabrata* species (7, 10–12)

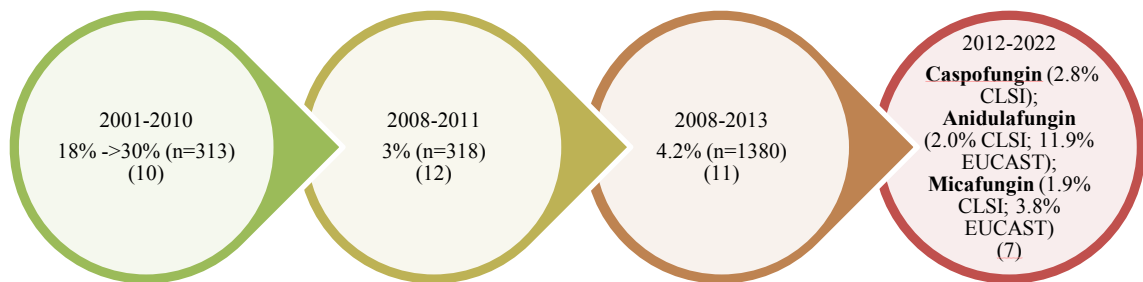


Figure 3. Distribution of Echinocandin resistance to *C. glabrata* (7, 10–12)

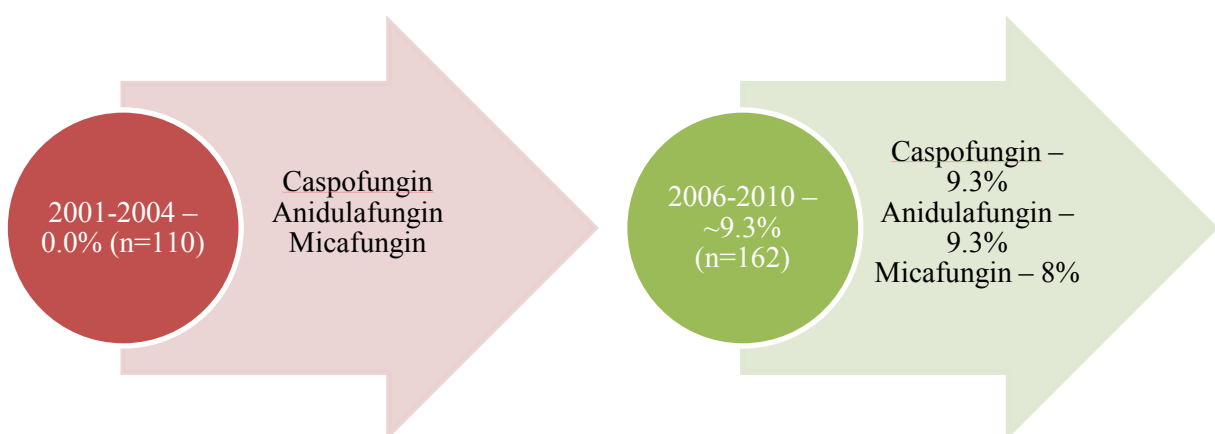


Figure 4. Distribution of a combination of Echinocandin resistance to *C. glabrata*, according to Alexander et al., 2013 (10).

Treatment

Based on the Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America, the first line of antifungal treatment is with Azoles group, mainly Fluconazole. It is necessary to apply therapy with azoles, only after determining the antimicrobial sensitivity of the isolated *C. glabrata* strain. It is therefore often recommended Fluconazole at a higher dose (800 mg daily, 12 mg/kg) for the treatment of invasive candidiasis caused by a susceptible strain of *C. glabrata* (9). In the event of unsuccessful treatment or azole resistance, it is recommended that echinocandins are used (2). On the other hand, a thorough investigation and history taking of the patient for previous therapy with echinocandins is imperative, since a treatment lasting 7 days is a prerequisite for the development of resistance in *C. glabrata* (13).

The emergence of multidrug-resistant strains of *Candida glabrata* poses a significant clinical challenge, as it limits the available treatment options and increases the risk of treatment failure and patient mortality. Therefore, understanding the mechanisms of antifungal resistance in *Candida glabrata* and implementing appropriate surveillance measures and treatment strategies are essential for effectively managing infections caused by this opportunistic pathogen. In cases of *C. glabrata* multidrug-resistant (MDR), combined antimicrobial therapy holds promise as a treatment strategy.

Amphotericin B (AmB) lipid formulations have effectively treated multidrug-resistant *C. glabrata*, which is resistant to both azoles and echinocandins (4). Doses up to 1 mg/kg daily should be considered for invasive *C. glabrata* infections. The typical dosage for AmB lipid formulation is 3–5 mg/kg daily when used for invasive candidiasis (9).

Flucytosine can be used for the treatment of invasive candidiasis in combination with another antifungal agent because of the high rate of emergence of resistance when used as monotherapy. This is probably why EUCAST does not recommend Flucytosine AST (14).

A new opportunity is also emerging (a new echinocandin) – resafungin with once-weekly dosing and activity against *Candida spp.*, including subgroups of echinocandin-resistant *Candida auris*, has recently been developed and is currently in phase III trials. It is approved by the FDA and the EMA as first-line drugs for invasive candidemia, esophageal candidiasis, and prevention of mycoses in transplant patients, which undoubtedly confirms their high utility in the fight against mycoses (4, 15).

The therapeutic approach is based on EUCAST's recommendations for infections caused by *C. glabrata* (16).

Azole:

- **Fluconazole** – increased dose: 800 mg x 1 time per day i.v. or 12 mg/kg for 6 weeks (9).

- **Itraconazole** – 200 mg 2 times per day for the first day, followed by 400* mg iv.

*Daily doses up to 200 mg x 2 times daily may be given depending on the infection. Capsules have 30% lower bioavailability than the oral solution.

- **Voriconazole** – in adults, the recommended oral dosing regimen for candidiasis includes a loading dose of 400 mg (6 mg/kg) twice a day for 2 doses, followed by 200–300 mg (3–4 mg/kg) twice a day.

Echinocandins:

- **Anidulafungin** – standard dose: 200 mg x 1 on the first day followed by 100 mg x 1 time daily

- **Caspofungin** – standard dose 70 mg x 1 on the first day followed by 50* mg x 1 (for weight ≤ 80 kg) and 70 mg x 1 (for weight > 80 kg)

- **Micafungin** – standard dose: 100 mg x 1 (for weight > 40 kg) or 2 mg/kg x 1 time daily, for patients weighing < 40 kg.

- Increased dose: 200 mg x 1 (for weight > 40 kg) or 4 mg/kg x 1 for patients weighing < 40 kg.*

*Increased dosage is indicated in patients who do not respond to the standard dosage.

Echinocandins for 2 weeks followed by fluconazole for at least 4 weeks is recommended (9).

Amphotericin B Formulas:

- **Liposomal Amphotericin B** – standard dose: 3 mg/kg x 1 time per day. In specific situations, increased doses up to 7 mg/kg.

- **Amphotericin B deoxycholate** – standard dose: 1 mg/kg x 1 time per day

- **Amphotericin B as a lipid complex (ABLC)** – 5 mg/kg x 1 time per day

Our experience in the Laboratory of Microbiology at the University Hospital “St. George” – Plovdiv shows that *C. glabrata* isolated from invasive infections is a rare occurrence. In three years from 2020 until 2023, we have identified three *C. glabrata* isolates from haemoculture that were AST. Consistent with the documented rates of resistance to the Azole group in scientific literature, two of our isolates exhibited resistance to itraconazole.

The growing prevalence of resistance in *C. glabrata* that is being documented in the USA serves as a warning to be vigilant about the potential spread of this strain in our region. The ease of travel across open borders in modern times may contribute to the rapid spread of this pathogenic fungus, which is highly adaptable to current treatment methods. This, in turn, poses a significant challenge for effectively treating the infection. In order to evaluate the epidemiological spread and gather statistical data for the Bulgarian region it is necessary to conduct microbiological surveys.

In conclusion,

1. There are no particular differences in the diagnosis of invasive and non-invasive *C. glabrata*. Diagnostic methods and the AST provide indispensable information for the correct treatment of the infection. Empiric therapy has led to levels of resistance.
2. There is a general increase in reported antifungal resistance.
3. The rising antifungal resistance necessitates the discovery of novel therapeutic approaches and markers that distinguish invasive from superficial candidiasis.
4. It seems that there are not enough studies on the antifungal resistance of *C. glabrata* in the European region, given that most of our sources stem from the US.
5. A significant proportion of the strains that are resistant to echinocandins also exhibit resistance to azoles, leaving polyenes as the only remaining treatment option.

Bibliography

1. Quindós G. Epidemiology of candidaemia and invasive candidiasis. A changing face. Rev Iberoam Micol. 2014 Jan;31(1):42–8.
2. Naicker SD, Shuping L, Zulu TG, Mpembe RS, Mhlana M, Tsotetsi EM, et al. Epidemiology and susceptibility of *Nakaseomyces* (formerly *Candida*) *glabrata* bloodstream isolates from hospitalised adults in South Africa. Med Mycol. 2023 Jun 5;61(6).
3. Frías-De-León MG, Hernández-Castro R, Conde-Cuevas E, García-Coronel IH, Vázquez-Aceituno VA, Soriano-Ursúa MA, et al. *Candida glabrata* Antifungal Resistance and Virulence Factors, a Perfect Pathogenic Combination. Pharmaceutics. 2021 Sep 22;13(10):1529.
4. Barantsevich N, Barantsevich E. Diagnosis and Treatment of Invasive Candidiasis. Antibiotics. 2022 May 26;11(6):718.
5. Pfaller MA, Rhomberg PR, Messer SA, Jones RN, Castanheira M. Isavuconazole, micafungin, and 8 comparator antifungal agents' susceptibility profiles for common and uncommon opportunistic fungi collected in 2013: temporal analysis of antifungal drug resistance using CLSI species-specific clinical breakpoints and proposed epidemiological cutoff values. Diagn Microbiol Infect Dis. 2015 Aug;82(4):303–13.
6. Berkow E, Lockhart S. Fluconazole resistance in *Candida* species: a current perspective. Infect Drug Resist. 2017 Jul;Volume 10:237–45.

7. Chesdachai S, Yetmar ZA, Ranganath N, Everson JJ, Wengenack NL, Abu Saleh OM. Antifungal Susceptibility Pattern of *Candida glabrata* from a Referral Center and Reference Laboratory: 2012–2022. *Journal of Fungi*. 2023 Aug 3;9(8):821.
8. Oxman DA, Chow JK, Frendl G, Hadley S, HersHKovitz S, Ireland P, et al. Candidaemia associated with decreased in vitro fluconazole susceptibility: is *Candida* speciation predictive of the susceptibility pattern? *Journal of Antimicrobial Chemotherapy*. 2010 Jul 1;65(7):1460–5.
9. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*. 2016 Feb 15;62(4):e1–50.
10. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R, et al. Increasing Echinocandin Resistance in *Candida glabrata*: Clinical Failure Correlates With Presence of FKS Mutations and Elevated Minimum Inhibitory Concentrations. *Clinical Infectious Diseases*. 2013 Jun 15;56(12):1724–32.
11. Pham CD, Iqbal N, Bolden CB, Kuykendall RJ, Harrison LH, Farley MM, et al. Role of FKS Mutations in *Candida glabrata*: MIC Values, Echinocandin Resistance, and Multidrug Resistance. *Antimicrob Agents Chemother*. 2014 Aug;58(8):4690–6.
12. Cleveland AA, Farley MM, Harrison LH, Stein B, Hollick R, Lockhart SR, et al. Changes in Incidence and Antifungal Drug Resistance in Candidemia: Results From Population-Based Laboratory Surveillance in Atlanta and Baltimore, 2008–2011. *Clinical Infectious Diseases*. 2012 Nov 15;55(10):1352–61.
13. Shields RK, Nguyen MH, Press EG, Updike CL, Clancy CJ. Caspofungin MICs Correlate with Treatment Outcomes among Patients with *Candida glabrata* Invasive Candidiasis and Prior Echinocandin Exposure. *Antimicrob Agents Chemother*. 2013 Aug;57(8):3528–35.
14. Barchiesi F, Arzeni D, Caselli F, Scalise G. Primary resistance to flucytosine among clinical isolates of *Candida* spp. *Journal of Antimicrobial Chemotherapy*. 2000 Mar;45(3):408–9.
15. Szymański M, Chmielewska S, Czyżewska U, Malinowska M, Tylicki A. Echinocandins – structure, mechanism of action and use in antifungal therapy. *J Enzyme Inhib Med Chem*. 2022 Dec 31;37(1):876–94.
16. European Committee on Antimicrobial Susceptibility Testing. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs for antifungal agents. 2020.

Atypical mycoplasmal pneumonia – a case study

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Pneumonia is an infection of the lower respiratory tract that involves the airways and parenchyma, with consolidation of the alveolar spaces. The causative agents may be viruses, bacteria, fungi. Atypical pneumonias are most often caused by intracellular microorganisms and are characterized by a milder and “atypical” clinical picture. Atypical pneumonia describes patterns typically more diffuse or interstitial than lobar pneumonia. The causative agents may be: *Chlamydia pneumoniae*, *Chlamydia psittaci*, *Mycoplasma pneumoniae* and viruses. *Mycoplasma pneumoniae* is most common after age 5 and is most prevalent in late summer and fall. We present a clinical case of a 13-year-old boy with fever, cough and pain in the left thoracic region. After physical examination a mildly hyperemic throat has been established. The rest of the parameters of the physical examination were normal. On radiography a right upper lung paramediastinal oval formation with a heterogeneous structure and mortared X-ray density with unsharp outlines and reduced transparency of the adjacent lung parenchyma was found. On CT scan, consolidation of the parenchyma of the second and third segments of the right lung was found. On ultrasonography, evidence of pneumonia was detected. PCR of sputum proved the causative agent – *Mycoplasma pneumoniae*. This case illustrates the difficulty in diagnosing atypical pneumonias. Knowledge of the most common causative agents of atypical pneumonias is critical for making the correct diagnosis and choosing the right treatment.

Keywords: *atypical pneumonia, Mycoplasma pneumoniae, consolidation, oval formation*

Introduction

Pneumonia is one of the most common causes of childhood hospitalization, with an incidence of 1.4 per 10,000 per year, in children 0–16 years. *Mycoplasma pneumoniae* is one of the most common causes of community-acquired pneumonia in children over 5 years of age. It is responsible for about 11% of respiratory infections in children under 14 years of age and about 21% in children around 5 years of age. In most cases, mycoplasma pneumonia is mild, but cases have been reported in which it progresses to fulminant necrotizing pneumonia, bronchiolitis obliterans, ARDS, pulmofibrosis, and other extrapulmonary complications (1). Coinfection or superinfection is common in mycoplasmal pneumonia, most commonly with *Streptococcus pneumoniae* and *Haemophilus influenzae*. Atypical pneumonias often proceed in a shrouded fashion, preceded by upper respiratory tract symptoms. Symptomatology, physical examination, and imaging findings may resemble various diseases, leading to some difficulties in making

the diagnosis. Making the correct diagnosis and proving the causative agent of atypical pneumonia is crucial for proper and timely treatment and prevention of complications.

Case presentation

A 13 year old boy with normal development presents with fever up to 39 °C, cough and right chest pain for 5 days. He was treated as an outpatient with Cefixime – no effect. He presented in unsatisfactory general condition, pale pink skin with preserved turgor and elasticity. No rash units on the body. Throat – slightly hyperemic. Lymph nodes – not palpable. The pulmonary status revealed symmetrical chest, shortened percussive tone in the right apex, vesicular breathing without wheezing. Examination of the cardiovascular system showed clear heart tones and no pathological noises, HR – 87/min, BP – 105/60 mmHg. Abdomen – soft, palpable, painless, without hepatosplenomegaly. Urinalysis – bilateral negative. The neurological status was age appropriate. The child was born from a third

normal pregnancy, at term, by a normal mechanism. Weight at birth – 4000 g and height – 51 cm. With normal physical and psycho-motor development and has been immunized according to calendar. Past illnesses – Varicella, Covid 19, frequent bronchobstructions from 4 y. o. to 7 y. o. Family history – mother with Hashimoto's thyroiditis, thalassemia, grandmother with diabetes mellitus and arterial hypertension.

Laboratory findings

From the paraclinical examinations there were no abnormalities in the full blood count, increased SED rate – 27 mm/ h and decreased APTT – 24.4 sec. The rest of the parameters were normal, no abnormalities from the biochemical examination.

A blood gas analysis revealed – pH – 7.42, pCO₂ – 40 mmHg, pO₂ – 73 mmHg, sO₂ – 95, ABE – 2, HCO₃ – 26. Functional respiratory study shows mild restrictive ventilatory disorder.

Mycoplasma pneumoniae was detected by PCR in microbiological examination.

Diagnostic imaging

Radiograph shows oval strong shadowing with heterogeneous structure and indistinct outline in the right upper lung paramediastinal field, lung parenchyma adjacent – with reduced transparency and enhanced drawing with marked small interlobe. A chest CT scan revealed consolidation of the parenchyma of segments II and III of the right lung. Echocardiography showed no structural or functional changes, echographic evidence of pneumonia was detected in the right parasternal. The abdominal ultrasonography finding was normal.

Treatment

Treatment with Ceftriaxone 2×2 grams and Clarithromycin 2×500 mg was started

and resulted in significant improvement. Treatment is continued on an outpatient basis with Clarithromycin – 2×500 mg for 10 days.

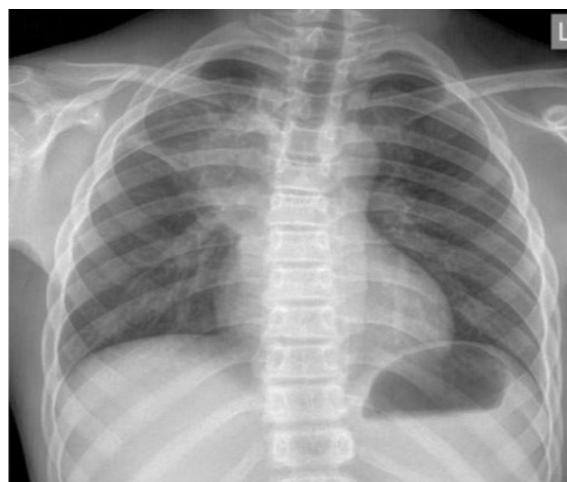


Figure 1. Chest X-ray



Figure 2. Chest CT-scan

Discussion

M. Pneumoniae is the most common causative agent of tracheobronchitis in pre-school and school-aged children (1). It is rarely a cause of hospitalization in children. The clinical picture most often includes febrility, astheno-adynergia, sore throat and dry cough (2). The special feature in the presented clinical case is the absence of any physical findings on examination and the unusual finding on X-Ray. The chest pain suggests pleural involvement. The presence of parapneumonic complications of mycoplasmal pneumonia is not uncommon. Their

incidence ranges around 20–25% (3). Parapneumonic effusion occurs by direct invasion of pathogens into the pleura through the alveolar airspace. There, they interact with mesothelial cells in a similar manner as with the respiratory epithelium and induce an inflammatory response. The clinical picture, laboratory and imaging studies give a wide scope for differential diagnosis. In the differential diagnostic plan, pneumococcal pneumonia, tuberculosis, mediastinum formation are discussed. The absence of elevated fibrinogen and the radiographic and clinical findings exclude the diagnosis. A PPD was performed, which showed a normoergic reaction, after which sputum was collected for a Zill-Nielsen test, which did not detect mycobacteria. Thus, the diagnosis of tuberculosis was rejected. Initially, the radiographic findings pointed to a process in the mediastinum, lymphoma, but after a CT scan was performed, this was ruled out. Furthermore,

the lack of lab data also rules out the diagnosis of lymphoma.

Conclusion

In the last year, cases of atypical pneumonia with the causative agent *M. Pneumoniae* are becoming more common, mainly in the age group of children over 5 years. It is rarely the cause of hospitalization. The clinical picture, laboratory and imaging studies give a wide scope for differential diagnosis. Correct and quickly placed diagnosis provides timely and adequate treatment and prevention of complications.

Bibliography

1. Mycoplasma Pneumoniae-Associated Necrotizing Pneumonia in Children : a case-report Chan ho Lee, So Yoon Jo, Keon Woo Na, Sung Won Kim, Yoon Ha Hwang Department of Pediatrics, Busan St. Mary's Hospital, Busan, Korea
2. Oski's Pediatrics Principles and Practice, 4th edition
3. Nelson's Essentials of Pediatrics 9ed

Preschool children and pulmonary function testing. Is the FOT (forced oscillation technique) the new option for better diagnostics?

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Introduction: The diagnostics and follow-up of the obstructive lung diseases in young children under 6 years of age have always been a challenge. The Forced oscillation technique (FOT) is effortless alternative of spirometry and is proven to be sensitive method of testing. **Aim:** The purpose of this study is to demonstrate the success rate of FOT compared to the traditional Spirometry in preschool age. **Materials and methods:** 20 healthy children aged 3–6 were enrolled in this study. They performed both FOT (RESMON PRO) and Spirometry (Vitalograph) test. **Results and discussion:** The forced oscillation technique applies an external oscillatory signal in order to determine the response of the respiratory system. It requires only tidal breathing and a total of three technically acceptable measurements, whereas the spirometry test needs a lot of instructions, effort and time. All 20 healthy patients tried to perform FOT and Spirometry in order to evaluate the success rate of the two examinations. All children at the age of 3 couldn't perform the spirometry, and the total success rate for spirometry was no more than 50%. In comparison near 90% performed FOT successfully. Some children couldn't execute the FOT due to two main reasons: crying out of fear and laughing. The child's laughter was mainly caused by the oscillations which create a tickling sensation in the child's oral cavity and chest. **Conclusion:** The technique of forced oscillations is an extremely effective, effort-independent and informative method in the diagnosis of obstructive diseases, even at the young age of less than 6 years.

Keywords: Forced oscillation technique, spirometry, airway obstruction, children

Introduction

The diagnostics and follow-up of obstructive lung diseases in preschool children under 6 years of age poses a major challenge for pulmonologists and pediatricians. A number of studies report that the highest average annual asthma physician office visits, hospitalizations and emergency department visits are much more in the preschool age compared to other age groups (1). (Figure 1)

Therefore, it is necessary to introduce new methods in the diagnostics of asthma that carry a highly informative value and are easy to be performed by preschool patients.

The golden standard for the diagnosis of bronchial asthma is spirometry, but this method is effort-dependent and difficult to perform in preschool age.

The ideal pulmonary function test (PFT) in preschool children should be applicable at any age to allow longitudinal study and monitoring of pulmonary function from infancy to adulthood, be easy to perform, safe, reproducible, sufficiently sensitive in early

disorders and in distinguishing sick from healthy children and finally be acceptable to the children and their parents. The Forced Oscillation Technique (FOT) is a new alternative to spirometry and has the potential to be a better and sufficiently informative method.

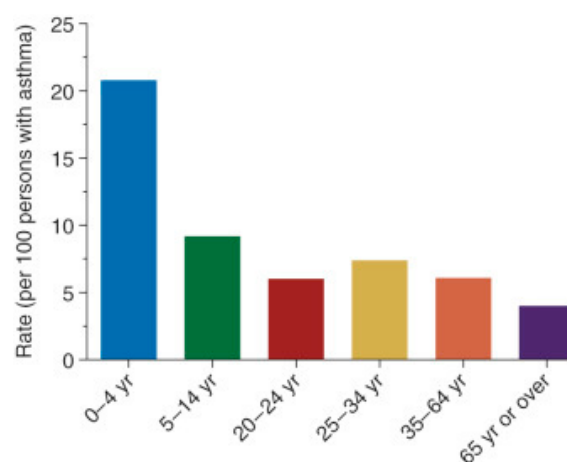


Figure 1. Emergency department visits for acute wheeze (risk-based) for the United States. (Data are the age-specific average annual rates for 2007–2008, from the U.S. National Surveillance of Asthma report).

Source: Moorman JE, Akinbami LJ, Bailey CM. National surveillance of asthma

Aim

The aim of this study is to prove the success rate and efficiency of this new method for Bulgaria – FOT and compare it with the routine spirometry method.

Materials and methods

The study involved 20 pulmonary healthy children between the ages of 3 and 6, who were examined using the spirometry (Vitalograph) and FOT (RESMON PRO) methods.

All 20 healthy patients were asked to perform FOT and Spirometry in order to evaluate the success rate of the two examinations. All children at the age of 3 couldn't perform the spirometry, and the total success rate for spirometry was no more than 50%. In comparison nearly 90% performed FOT successfully. Some children couldn't execute the FOT due to two main reasons: crying out of fear and laughing. The child's laughter was mainly caused by the oscillations which create a tickling sensation in the child's oral cavity and chest.

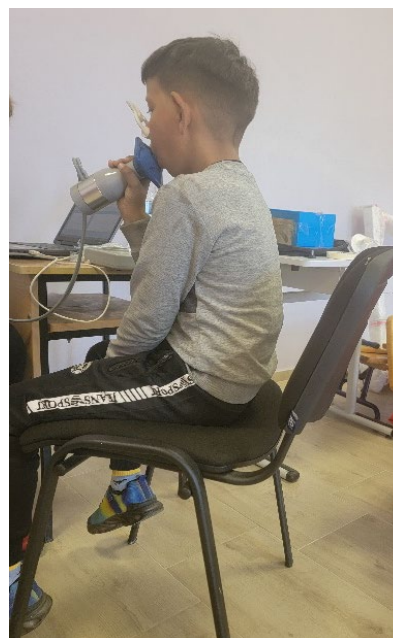
Discussion

Spirometry is a non-invasive method for researching the function of breathing, including measurement of volume and speed parameters. It is the most common of the pulmonary function tests (PFT) and provides information on a wider range of parameters, portable devices can be used for it, also the method is affordable (2).

The disadvantages of spirometry are that the quality of the study depends on the patient's efforts, as well as on his level of cooperation, the results can easily be obscured by artifacts due to difficulties in the implementation of some of the tested parameters (for example, FEV1).

During the test the patient is asked to put on nose clips to prevent air escape and a

breathing sensor in their mouth forming an airtight seal. Guided by a technician, the patient is given step by step instructions to take a deep maximum effort inhale, followed by an abrupt maximum effort exhale lasting for at least 6 seconds (4 seconds for children). When assessing possible upper airway obstruction, the technician will direct the patient to make an additional inhalation to complete the round. The timing of the second inhale can vary between patients depending on the length of the proceeding exhale. In some cases, each round of the test will be proceeded by a period of normal breathing for additional data (Picture 1).



Picture 1. A 6-year-old boy performing spirometry with Vitalograph.

Spirometry, especially the forced exhalation, has proven to be very difficult for certain groups of people: preschool children, elderly people, patients examined during the asthma attack, patients with neuromuscular disorders and minority groups. In our study we also observed that the success rate for children between 4–6 years old was around 50%, whereas all children under the age of 4 couldn't perform the test.

The forced oscillation technique (FOT) is a non-invasive method for assessing respiratory tract resistance and respiratory mechanics during tidal breathing.

Although not as common as spirometry, this method can be performed by preschool children and patients unable to perform quality spirometry.

FOT is a method in which the impedance of the respiratory system is measured by superimposing small-amplitude pressure oscillations on the respiratory system and measuring the resultant oscillatory flow.

The patient is connected via a mouthpiece to the set-up. It most commonly consists of a loudspeaker to deliver the forced oscillatory signal. Usually, a total of three to five measurements should be performed (3) (4).

The subject is placed in a sitting position with the head in a neutral or slightly extended position. Flexion of the head should be avoided. During the measurement, the subject (or technician) firmly supports his/her cheeks and the floor of the mouth using both hands and a nose clip. The test lasts less than 1 minute and needs 6 accepted breaths (Picture 2).



Picture 2. A 6-year-old girl performing FOT with REMON PRO device.

The main advantage of FOT is that it requires minimal cooperation of the patient, and the duration of the examination is significantly shorter. The indicator resistance provides information of the degree of obstruction, similar to FEV1 in spirometry, but is performed with tidal breathing, and reactance shows how well the distal parts of the bronchial tree are ventilated and respectively – peripheral obstruction. The fact that it is independent of effort is a key quality of the FOT method in difficult-to-cooperate patients such as preschool children. In addition, the methodology solves the problem with the interpretation of peripheral obstruction indicators FEF75%, FEF50%, FEF25% – 75% (forced expiratory flow rates between 25% and 75% of expiration), which are significantly dependent on volume and are not applicable in cases of an incomplete forced maneuver (5) (6).

On the other hand, difficulties are sometimes experienced in distinguishing an obstructive from a restrictive pulmonary defect; additional data is needed to determine reference values for children in the population of Bulgaria; trained medical staff and expensive apparatus are required.

All these characteristics of FOT make the method suitable for all age groups. Our study shows that almost all children are able to perform the test and the only cases in which preschool children fail are when they are very scared and cannot stop crying and when they are laughing or making sounds in the tube. Allowing the parents to accompany the patients in the lung function laboratory improves cooperation. They can also be given pieces of equipment to train their child at home in order to encounter further difficulties.

Conclusion

The introduction of more innovative and modern methods in the practice of the Bulgarian doctors to protect children's health is extremely necessary. The forced oscillation technique provides a new opportunity for prevention, early diagnostics, treatment and control of respiratory diseases in preschool children and is a chance to significantly improve the quality of life of patients.

References

1. Moorman JE, Akinbami LJ, Bailey CM. National surveillance of asthma: United States, 2001-2010. <https://pubmed.ncbi.nlm.nih.gov/24252609/> Vital Health Stat 3 2012. At
2. Lazova S. Pulmonary function tests in the new realities [Bulgarian] InSpiro September 2021 pp16-22
3. Lazova, S. Spirometry - known and unknown [Bulgarian] InSpiro September 2021 pp 23-30
4. Sabine C. Zimmermann et al. Day-to-day variability of forced oscillatory mechanics for early detection of acute exacerbations in COPD Eur Respir J 2020; 56: 1901739
<https://doi.org/10.1183/13993003.01739-2019>
5. Alice M. Cottee et al. Bronchodilator Response Assessed by the Forced Oscillation Technique Identifies Poor Asthma Control with Greater Sensitivity Than Spirometry American College of Chest Physician Published by Elsevier
doi: <https://doi.org/10.1016/j.chest.2019.12.035>
6. Afaf Alblooshi et al. Is forced oscillation technique the next respiratory function test of choice in childhood asthma World J Methodol. doi: 10.5662/wjm.v7.i4.129

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Acute myeloid leukemia diagnosed during pregnancy: a case report

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Introduction: Acute myeloid leukemia (AML) occurring during pregnancy is not a common medical event. Its treatment is significant clinical challenge, regarding the therapy-associated risks both for the mother and the fetus and in addition raises a serious question whether the pregnancy should be continued. AML is often complicated with infection, bleeding and placental dysfunction. On the other hand all cytostatic agents are teratogenic therefore maternal health must be paramount. **Case presentation:** We report a case of 28-year-old pregnant female in 14-week of gestation presenting with classical findings of AML proven by the presence of blasts cells in peripheral blood and bone marrow. Flowcytometry confirmed AML. Abortion on medical indications was performed in consideration of the progressive course of the disease. Standard induction "7+3" and consolidation chemotherapy HDAC were administered and the patient achieved hematological remission. Donor search found HLA – matched unrelated donor. The patient underwent allogeneic stem – cell transplantation followed by donor lymphocyte infusion and a second stem cell transplant from the same donor because of progressive evolution of the disease. Unfortunately AML relapsed again after second transplant and the patient died. **Discussion and conclusion:** Simultaneous establishment of pregnancy and life-threatening diagnosis such as AML requires individual approach. Evidence obtained so far are insufficient for determining a causative association between pregnancy and hematological cancers. Many questions are still open regarding the cessation of pregnancy, the possibility to preserve the fetus and postpone chemotherapy and the option for cord blood stem cell collection.

Keywords: Acute myeloid leukemia (AML), Pregnancy, Abortion on Medical Indication

Introduction

Acute myeloid leukemia (AML) occurring concomitantly with pregnancy is a rare medical event. The incidence of AML is estimated to be between 1 in 75,000 and 100,000 cases.¹ AML is often complicated by infection, bleeding and placental dysfunction.² It would be beneficial to consider a number of key questions regarding the therapeutic management of this condition. These include whether it is possible to postpone treatment following delivery, how to administer chemotherapy in a manner that is both safe for the mother and minimizes the risk of teratogenic side effects for the foetus, and whether a medical abortion should be carried out on medical indications because the health of the mother must always be the paramount concern.

Case presentation

We present a case of a 28-year-old pregnant woman in her 14th week of pregnancy

who manifested with a throbbing right occipital headache of several weeks' duration. Prophylactic tests indicated the presence of macrocytic anaemia, low HbG, apparent leukocytosis and thrombocytopenia. She was referred to a haematologist and admitted to the clinic for further diagnostic investigation. On examination, the patient is in good general condition, is pregnant in the 14th week of pregnancy and is afebrile, except for skin and visible mucous membranes which were pale. All other findings were unremarkable. The CBC data indicated the presence of macrocytic anemia, with notable leukocytosis and thrombocytopenia: HGB – 71 g/L, RBC – $1.89 \cdot 10^{12}/L$, MCV – 112.1 fl, MCH – 37.7 pg, WBC – $19.67 \cdot 10^9/L$ with 50% blast cells, PLT – $25 \cdot 10^9/L$. The biochemical panel demonstrated serum glucose within the reference ranges and no evidence of liver or biliary dysfunction, normal coagulogram. CRP – 30.6 mg/L was elevated, as was the biomarker for cell and tissue destruction

LDH – 966 U/L. Further diagnostic hematological investigations were performed, including cytochemistry, bone marrow biopsy, flow cytometry and molecular genetic testing. The myelogram indicated the presence of total metaplasia of predominantly granular myeloid blasts with metachromasia, which appeared to be partially differentiated with multiple mitoses. Immunophenotyping by flow cytometry revealed positive cluster of differentiation (CD) markers of myeloid lineage with respect to structural subtyping. The positive markers corresponded to the criteria for acute myeloid leukemia: the results were as follows: 40% blast population with aberrant myeloid phenotype: CD13/+; CD33/+; CD71/+; CD45/+; CD38/+; HLA DR/+; CD117/+.² Molecular genetic testing revealed the following: balanced t(8;21)/AML-ETO (+) /fusion protein; FLT3-ITD (+)/internal tandem duplication mutations in the tyrosine kinase receptor. Based on the comprehensive analysis of the available data, the patient was diagnosed with AML t(8;21)/AML-ETO (+); FLT3-ITD(+), which is classified as a high risk profile.² Meanwhile, the patient underwent a consultative gynecological examination and was presented to the institution's Clinical Oncohaematology Committee, which decided to terminate the pregnancy in view of the progressive nature of the disease and the impending chemotherapy, which is highly teratogenic to the foetus. Abortion was performed on medical indications. The patient underwent standard induction treatment “7+3” and consolidation with HDAC. Disease assessment by flowcytometry showed MRD (-) bone marrow, but and genetic testing found AML-ETO/+and FLT3-ITD/+ which were initially positive. The patient was referred for haploidentical allogeneic stem cell transplantation especially because of FLT3-ITD mutation poor outcomes predictions³. The donor was her father. A

clinical re-evaluation was performed prior to transplantation, which yielded the following results: flow cytometry revealed no pathological populations; myelogram demonstrated a normal blood series with myeloblasts less <3%; molecular genetic testing still indicated the initially detected transcripts: AML-ETO/+and FLT3-ITD/+. Donor-specific anti-HLA antibodies were present. A desensitizing protocol involving Rituximab at a dose of 375 mg/m² and intravenous immunoglobulin (IVIG) at a dose of 6 g per day was implemented. Following the desensitising regimen, a myeloablative conditioning protocol Fludarabine/Busulfan/Anti-Thymocyte Globulin (Flu/Bu/ATG) was administered + allogeneic transplant of 4.74.10⁶/kg CD34+ cells. The only early posttransplant complications were aphthous stomatitis, folliculitis, and diarrhoeal syndrome, which were effectively managed. Engraftment was registered on day +21 for neutrophils and on day +24 for platelets. Full donor chimerism was registered on day +72, and was maintained for 6 months but on month 12, AML relapsed with 40% myeloblasts in the bone marrow, FLT3+. Targeted therapy with Gilterinib 120 mg/d was started with no effect. Chemo-DLI – Azacitidine was tried but no remission was induced either. The patient was referred for second second haploidentical allogeneic transplant from the same donor after Bu/Aza conditioning regimen. Remission of AML was not achieved. The patients condition deteriorated with gastrointestinal tract bleeding, hematemesis, melena, hypotension, and circulatory collapse, necessitating the initiation of a full-scale resuscitation effort. However, this proved to be unsuccessful. The patient's condition deteriorated, resulting in a death.

Discussion

Malignancy of any origin is diagnosed in approximately 0.1% of pregnancies,

representing the second leading cause of maternal mortality, after gestation-related vascular complications. Amongst the various types of cancer, haematological malignancies are the most commonly observed in pregnant women, with predomination of lymphomas followed by the group of leukemias. The incidence of haematological malignancies during pregnancy appears to be higher among women who became pregnant at an advanced age⁴. The majority of AML cases are reported in the second and third trimesters, with 40% of cases occurring in the second trimester and 60% occurring in the third trimester. In contrast, only 23% of cases occur in the first trimester.⁵ A multitude of physiological alterations occur throughout the process of pregnancy. These include an increase in plasma volume, the formation of a third space created by amniotic fluid, hepatic oxidation and renal clearance.⁶ These modifications influence the distribution, metabolism, and excretion of drugs. The vast majority of chemotherapeutic agents are categorized as Pregnancy Category D. It has been estimated that up to 16% of fetuses exposed to chemotherapy during the first trimester are born with a congenital malformation. Even in third trimester certain systems remain vulnerable, especially those involved in haematopoiesis and the central nervous system (CNS). Furthermore, tissue growth may be adversely affected. Exposure to chemotherapeutic agents during the second and third trimesters of pregnancy is associated with an increased risk of a number of adverse outcomes. This includes preterm delivery, fetal growth restriction, and intrauterine fetal demise⁷ Pregnancy cannot be considered a trigger for AML. This is despite the complexity of pregnancy physiology with respect to haematological parameters, including pregnancy-associated anaemia, decreased leukocyte levels and platelet

variations.⁸ In addition, the accepted etiological factors and risk factors for acute myeloid leukaemia (AML), including somatic mutations, recurrent mutant genes, and age-related epigenetic aberrations, are applicable in this context. Moreover, the immunosuppressive environment that characterizes pregnancy, primarily driven by the expansion of regulatory T cells, may also facilitate tumour progression. Nevertheless, there is currently a paucity of reliable animal models and robust clinical data that can confirm the involvement of these mechanisms in pregnancy-associated haematological cancers.⁶

The most advisable course of action is abortion on medical indications, particularly in the first trimester, as the use of chemotherapy in this period is known to result in the death of the foetus, in addition to the expected necessity of further intensive treatment, including stem cell transplantation, which is contraindicated during pregnancy. Data from individual institutional experience with AML cases show that the administration of induction therapy (daunorubicin and cytarabine) during the first trimester of pregnancy results in miscarriage, haemorrhagic complications and no successful delivery. During the second and third trimesters, cytoreductive therapy, although safer according to the literature, shows a controversial complete response rate (CR), but also frequent fatal recurrent infections, haemorrhagic events, despite which there are cases of premature delivery, most of them without evidence of congenital malformations.⁹

Conclusion

It is evident that the diagnosis of haematologic malignancy during pregnancy is a profoundly distressing event for all those concerned, including the patient, her relatives, and the medical team. Such a diagnosis

has implications for a number of areas, including therapeutic considerations, ethical issues, religious considerations and social factors. In the case of the simultaneous establishment of pregnancy and a life-threatening diagnosis, such as AML, an individual approach is required. The case presented is one of a multitude of medically challenging scenarios currently facing the scientific community. Nevertheless, the ambiguity and lack of validated therapeutic algorithms present a significant opportunity for future investigation. A multidisciplinary approach, involving specialists from various medical fields, is essential for the development of effective management strategies. A number of questions remain unanswered regarding the cessation of pregnancy, the possibility of preserving the fetus and postponing chemotherapy, and the option of cord blood stem cell collection.

References

1. Mahmoud HK, Samra MA, Fathy GM. Hematologic malignancies during pregnancy: A review. *J Adv Res.* 2016;7(4):589-96. doi: 10.1016/j.jare.2016.02.001
2. Kaushansky K, & Prchal J.T., & Burns L.J., & Lichtman M.A., & Levi M, & Linch D.C.(Eds.), Eds. Kenneth Kaushansky, et al. McGraw-Hill Education, 2021
3. Schiller, G. J., Tuttle, P., & Desai, P. Allogeneic hematopoietic stem cell transplantation in FLT3-ITD-positive acute myelogenous leukemia: the role for flt3 tyrosine kinase inhibitors post-transplantation. *Biology of Blood and Marrow Transplantation*, 2016; 22(6), 982-990.
4. Lishner M, Avivi I, Apperley JF, Dierickx D, Evens AM, Fumagalli M, Nulman I, Oduncu FS, Pecatori FA, Robinson S, Van Calsteren K, Vandembroucke T, Van den Heuvel F, Amant F. Hematologic Malignancies in Pregnancy: Management Guidelines From an International Consensus Meeting. *J Clin Oncol.* 2016;34(5):501-8. doi: 10.1200/JCO.2015.62.4445
5. Fracchiolla, Nicola & Sciumè, Mariarita & D'Ambrosi, Francesco & Guidotti, Francesca & Ossola, Manuela & Chidini, Giovanna & Gianelli, Umberto & Merlo, Daniela & Cortelezzi, Agostino. Acute myeloid leukemia and pregnancy: Clinical experience from a single center and a review of the literature. *BMC Cancer.* 2017; 17. 10.1186/s12885-017-3436-9.
6. Brenner B, Avivi I, Lishner M. Haematological cancers in pregnancy. *Lancet.* 2012;379(9815):580-7. doi: 10.1016/S0140-6736(11)61348-2
7. Albright CM, Wenstrom KD. Malignancies in pregnancy. *Baillière's Best Practice & Research Clinical Obstetrics & Gynaecology.* 2016; 33:2-18 doi: 10.1016/j.bpobgyn.2015.10.004.
8. Cunningham, F., Leveno, K., Bloom, S., Spong, C.Y. and Dashe, J. (2014) *Williams Obstetrics*. 24th Edition, McGraw-Hill, New York.
9. Greenlund LJ, Letendre L, Tefferi A. Acute leukemia during pregnancy: a single institutional experience with 17 cases. *Leuk Lymphoma.* 2001 May; 41(5-6):571-7. doi: 10.3109/10428190109060347

III. Pharmacy and Pharmacotherapy Session

Optimized hydrogel with tetracycline-loaded liposomes for treatment of bacterial skin infections

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Introduction: Skin infections are some of the most common medical conditions. The treatment of bacterial dermal infections includes the use of antibiotics, which can be administered orally or topically. Tetracyclines are often used in the therapy. **The aim** of the present study was to formulate a semi-solid gel form with tetracycline-loaded liposomes, for topical administration of tetracycline. **Materials and methods:** liposomes were prepared using egg-yolk phosphatidylcholine, cholesterol and Kolliphor P188, by thin-layer hydration technique, followed by extrusion, in order to obtain liposomes with appropriate size. Four models were prepared with varying concentrations of phosphatidylcholine, cholesterol, emulsifier and tetracycline. The formulated liposomes were characterized in terms of particle size, surface morphology, drug loading and encapsulation efficiency using dynamic laser scattering, scanning electron microscopy and UV-Vis spectroscopy. Semi-solid formulation was obtained using Carbomer 934, gelled with triethanolamine. *In vitro* drug release was performed via diffusion through dialysis membrane at $32\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$. The obtained results were used to determine the drug release kinetics. **Results and discussion:** The formulated vesicles were spherical in shape, showing relatively high drug encapsulation efficiency and suitable size at around 200 nm. After incorporation in the hydrogel, prolonged drug release was observed. Based on the achieved results, a promising formulation was proposed for topical treatment of bacterial skin infections.

Introduction

Skin disorders affect 20–30% of the world population. Biologic agents, including bacteria, fungi, viruses, and skin parasites can lead to severe cutaneous infections in occupational settings. Cutaneous bacterial infections are very common and are usually the result of a bacterial invasion near soft tissues. Most skin infections are caused by *Staphylococcus aureus* and group A β -haemolytic streptococci, with various manifestations and severity. Clinical manifestations can range from mild superficial lesions to life-threatening necrotizing and systemic infections [1]. Management is determined by the severity and location of the infection and by patient comorbidities. Uncomplicated superficial pyodermas are often treated with topical antimicrobial drugs. Parenteral antibiotic therapies have always been associated with several

limitations that could be overcome by nano-based drug delivery systems [2].

Tetracyclines are active against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. The favourable antimicrobial properties of these agents and the absence of major adverse side effects has led to their extensive use in the therapy of human and animal infections. It is well established that tetracyclines inhibit bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the bacterial ribosome [3].

Drug delivery systems (DDSs) offer the potential to enhance the therapeutic index of drugs by increasing the drug concentration, the residence time in target cells and minimizing the side effects. DDSs involve

delivering the potentially active drug to the site of action via a nano-vehicle to enhance the pharmacological properties of free drugs and cover their undesirable features through improving drug pharmacokinetics and bio-distribution, as well as acting as drug reservoirs [4, 5]. These nanoparticles (NPs) usually range from a few nanometres to several hundred nanometres according to their intended application. Different natural, organic and inorganic materials are used to create NPs including ceramic, polymers, metals, and lipids that generate systems like micelles and liposomes [6]. Liposomes are now considered one of the most commonly used nanocarriers for various potentially active hydrophobic and hydrophilic molecules due to their high biocompatibility, biodegradability, and low immunogenicity [7]. They proved to enhance drug solubility and control distribution. Their capacity for surface modifications is a prerequisite for achieving targeted, prolonged, and sustained release. Based on the composition, liposomes can be considered to have evolved from conventional, long-circulating, targeted, and immune-liposomes to stimuli-responsive and actively targeted liposomes [8].

Aim

The aim of the present study is to develop a semi-solid gel form, containing tetracycline-loaded liposomes, intended for dermal application.

Materials and methods

1. Materials

Egg-yolk phosphatidylcholine (60%, TLC), Cholesterol, Kolliphor P188 and

Tetracycline HCl were purchased from Sigma-Aldrich, Germany. Carbopol 940 was purchased from Fillab Ltd, Bulgaria. Other chemicals, used for the study, were of analytical grade.

2. Methods

2.1. Preparation of tetracycline loaded liposomes

Four models of liposomes were prepared with varying concentrations of phosphatidylcholine, cholesterol, Kolliphor P188 and tetracycline. The four models with the corresponding factors' levels are presented in *Table 1*.

Liposomes were prepared by thin-layer hydration method, which was the first described method, by Bangham et. al, for the preparation of liposomes [9]. Lipid compounds and surface-active ingredients were dissolved in a chloroform/methanol solution (9/1). The resulting solution was transferred to a round-bottom flask and the organic solvent was evaporated under reduced pressure at 40°C until a thin film was obtained on the bottom of the flask, using rotary evaporator (Rotavapor RII, Buchi, Germany). The thin layer was maintained under vacuum until the complete evaporation of the organic solvents. Furthermore, the lipid film was hydrated for 2 h with Phosphate buffer saline (pH 7.4), in which tetracycline was dissolved. The resulting suspension was stored at 4°C overnight for the complete formation of the vesicles. Subsequently, the multilamellar vesicles were subjected to a 9-cycle-extrusion process through a polycarbonate membrane with pore-size of 0.1 µm, after which the liposomes were characterized.

Table 1. Liposomal models with their varying factors.

Model	Phosphatidylcholine: Cholesterol	Kolliphor P188 (mg)	Lipid phase: Tetracycline
M1	3:1	50	4:1
M2	3:1	100	2:1
M3	2:1	50	2:1
M4	2:1	100	4:1

2.2. Characterization of surface properties

Average size and ζ -potential were characterized by Dynamic laser scattering method (Zetasizer Ultra, Malvern Instruments Ltd, United Kingdom). Briefly, liposomal suspension was diluted 1:10 with ultra-pure water to avoid multiple light scattering. Z-potential was determined through the electrophoretic mobility of the liposomes using the same apparatus.

2.3. Determination of entrapment efficiency (EE)

EE was determined indirectly, through analysis of the unincorporated drug. 2 mL of the liposomal suspension was ultracentrifuged (Vivaspin 6, 10 000 MWCO, Sartorius Stedim Lab ltd, United Kingdom) for 2 hours at 4284 RCF. After that, the free tetracycline was analyzed using UV-Vis spectroscopy (Evolution 300 UV-VIS, Thermo Fisher Scientific, USA) at wavelength $\lambda = 275$ nm. The entrapment efficiency was calculated using the following equation:

$$EE\% = \frac{W_{tot} - W_{free}}{W_{tot}} * 100, \text{ where}$$

W_{tot} – total amount of API;

W_{free} – unentrapped API.

2.4. Preparation of hydrogel.

Liposome-loaded hydrogel was prepared using Carbopol 940, dispersed at ultra-pure water at concentration of 1% (w/v) and the resulting suspension was stored at 4°C overnight, for the complete swelling of the polymer. Liposomes were added dropwise to the Carbopol suspension, under continuous stirring, in Carbopol:Liposomes ratio 5:1. The

suspension was gelled with the addition of the alkalizing agent triethanolamine [10].

2.5. *In vitro* dissolution test

In vitro dissolution assay was conducted through the dialysis bag method. The liposome-loaded gel was sealed in a dialysis tube (MCWO 12400) and placed in a phosphate buffer saline pH 7.4 at temperature $32 \pm 5^\circ\text{C}$ [11]. At defined time intervals, 2 ml samples were measured, using UV-Vis spectrophotometry (Evolution 300 UV-VIS, Thermo Fisher Scientific, USA) at wavelength $\lambda = 275$ nm, to quantitatively determine the tetracycline content.

Results and discussion

1. Surface characteristics and entrapment efficiency

The physical characteristics of the liposomes are presented in Table 2. There is a clear trend of increase in the average size with the increase of the cholesterol concentration, which is probably due to the inclusion of cholesterol in the membranes of the liposomes, making them thicker. At the same time there is a decrease in the average size of the liposomal formulations with the increase of the surface-active ingredient. Significant increase in the entrapment efficiency was observed as the concentration of tetracycline increases. Based on the obtained data model M2 was selected as optimal, due to its smallest average size and highest entrapment efficiency, which was further incorporated into a semi-solid gel form.

Table 2. Surface characteristics and entrapment efficiency of the prepared liposomal models

Model	Average size $\pm$ SD (nm)	Z-potential $\pm$ SD(mV)	Entrapment efficiency (%)
M1	209.2 $\pm$ 1.756	-35.84 $\pm$ 0.5128	33.39
M2	160.7 $\pm$ 1.536	-37.13 $\pm$ 0.7159	79.17
M3	258.1 $\pm$ 1.983	-36.04 $\pm$ 0.8124	71.79
M4	230.1 $\pm$ 2.094	-36.32 $\pm$ 0.6976	29.10

2. *In vitro* dissolution assay

Figure 1 presents the *in vitro* drug-release profile of tetracycline from the liposome-loaded gel. An extended drug release tendency is observed, as only 20% of the incorporated drug was released after 360 minutes. According to Verma et al., liposomes with size between 120 nm and 190 nm are accumulated in *Stratum corneum* region and barely penetrate in the viable epidermis and dermis, which will provide a significantly prolonged local effect [12].

The data obtained from the *in vitro* dissolution test was fitted in some of the mathematical models describing the drug release process. Table 3 shows the different models with their corresponding coefficients of determination (R^2). The highest R^2 -value was observed in the Baker-Lansdale model, which usually describes the drug release from a spherical particle. This fully correlates with the fact that the liposomes have a spherical structure [13].

Table 3. Mathematical models of drug release and their corresponding correlation coefficients (R^2)

Mathematical model	R^2 -value
Zero order	0.8624
First order	0.8796
Krossmeyer-Peppas model	0.8888
Hixon-Crowell model	0.8607
Higuchi model	0.9301
Baker-Lansdale model	0.9656

Conclusion

Based on the conducted study, it can be concluded that tetracycline can be successfully incorporated into a liposomal structure with a relatively high entrapment efficiency. The resulting tetracycline-loaded liposomes can be included into a semi-solid gel form, providing significantly extended drug release of tetracycline. The drug release process from the designed formulations can be mathematically described with a high degree of accuracy.

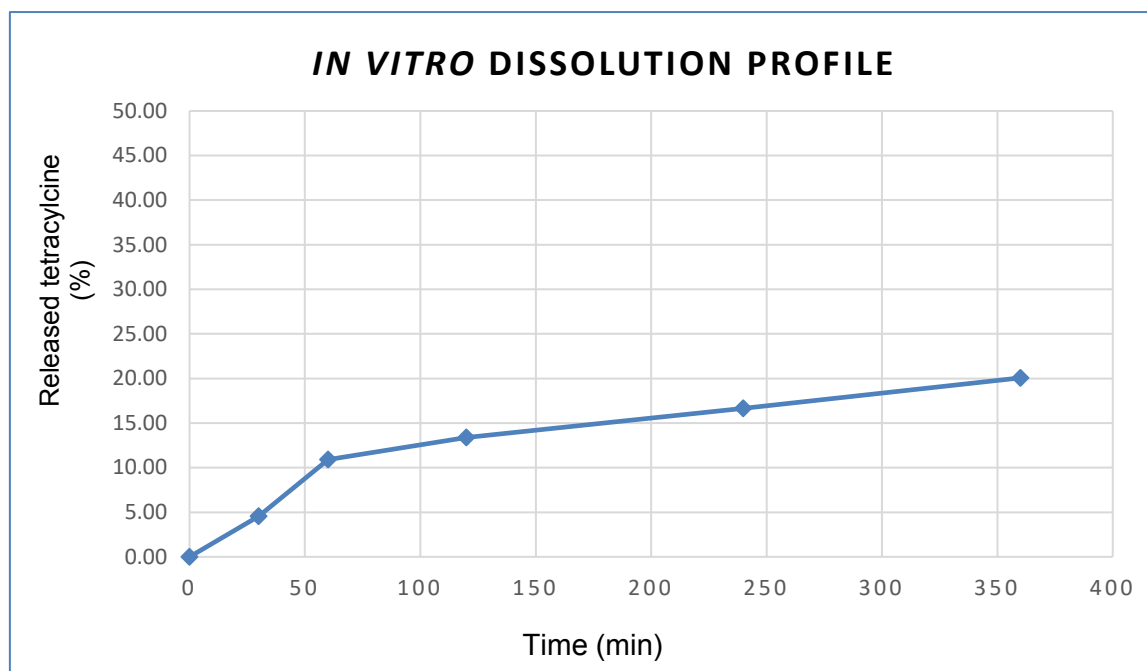


Figure 1. *In vitro* dissolution profile of tetracycline-loaded liposomes.

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References

1. Bessa, G. (2023). Bacterial Infections. In: Rangel Bonamigo, R. (eds) *Dermatology in Public Health Environments*. Springer, Cham. https://doi.org/10.1007/978-3-031-13505-7_9
2. Falcone, M., Concia, E., Giusti, M. et al. Acute bacterial skin and skin structure infections in internal medicine wards: old and new drugs. *Intern Emerg Med* **11**, 637–648 (2016). <https://doi.org/10.1007/s11739-016-1450-6>
3. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev.* 2001 Jun;65(2):232-60 ; second page, table of contents. doi: 10.1128/MMBR.65.2.232-260.2001. PMID: 11381101; PMCID: PMC99026.
4. Weiwei Gao, Yijie Chen, Yue Zhang, Qiangzhe Zhang, Liangfang Zhang, Nanoparticle-based local antimicrobial drug delivery, *Advanced Drug Delivery Reviews*, Volume 127, 2018, Pages 46-57, ISSN 0169-409X, <https://doi.org/10.1016/j.addr.2017.09.015>.
5. Ana Henriques Mota, Patrícia Rijo, Jesús Molpeceres, Catarina Pinto Reis, Broad overview of engineering of functional nanosystems for skin delivery, *International Journal of Pharmaceutics*, Volume 532, Issue 2, 2017, Pages 710-728, ISSN 0378-5173, <https://doi.org/10.1016/j.ijpharm.2017.07.078>.
6. Movaffagh, J., Nouroollahian, T., Khalatbari, S. et al. Fabrication of Zein-Chitosan-Zein Sandwich-Like Nanofibers Containing Teicoplanin as a Local Antibacterial Drug Delivery System. *J Pharm Innov* **18**, 911–922 (2023). <https://doi.org/10.1007/s12247-022-09686-2>
7. M Šentjurc, K Vrhovnik, J Kristl, Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method, *Journal of Controlled Release*, Volume 59, Issue 1, 1999, Pages 87-97, ISSN 0168-3659, [https://doi.org/10.1016/S0168-3659\(98\)00181-3](https://doi.org/10.1016/S0168-3659(98)00181-3).
8. Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine*, 10, 975–999. <https://doi.org/10.2147/IJN.S68861>
9. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *Journal of Molecular Biology.* 1965;13(1):238–252. doi: 10.1016/S0022-2836(65)80093-6.
10. A-sasutjarit, R., Sirivat, A. & Vayumhasuwan, P. Viscoelastic Properties of Carbopol 940 Gels and Their Relationships to Piroxicam Diffusion Coefficients in Gel Bases. *Pharm Res* **22**, 2134–2140 (2005). <https://doi.org/10.1007/s11095-005-8244-2>
11. Hua, S. (2014). Comparison of in vitro dialysis release methods of loperamide-encapsulated liposomal gel for topical drug delivery. *International Journal of Nanomedicine*, 9, 735–744. <https://doi.org/10.2147/IJN.S55805>
12. D.D. Verma, S. Verma, G. Blume, A. Fahr, Particle size of liposomes influences dermal delivery of substances into skin, *International Journal of Pharmaceutics*, Volume 258, Issues 1–2, 2003, Pages 141-151, ISSN 0378-5173, [https://doi.org/10.1016/S0378-5173\(03\)00183-2](https://doi.org/10.1016/S0378-5173(03)00183-2).
13. 5-Mathematical models of drug release, Editor(s): Marcos Luciano Bruschi, *Strategies to Modify the Drug Release from Pharmaceutical Systems*, Woodhead Publishing, 2015, Pages 63-86, ISBN 9780081000922, <https://doi.org/10.1016/B978-0-08-100092-2.00005-9>.

RP-HPLC method for simultaneous determination of paracetamol, diclofenac sodium and omeprazole in tablet dosage form

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Introduction: The combination of analgesics is a strategy to achieve therapeutic goals, such as facilitating patient compliance, simplifying prescribing, etc. Combination of nonsteroidal anti-inflammatory drugs with a proton pump inhibitor omeprazole is beneficial as it suppress gastric acid secretion. **Aim:** The aim of this study is to develop and validate simple, precise and accurate reverse-phase high performance liquid chromatographic (RP-HPLC) method with isocratic elution mode for simultaneous determination of paracetamol, diclofenac sodium and omeprazole in tablet dosage forms. **Materials and methods:** The chromatography was carried out on a C18, 250 mm x 4.6 mm, 5 μ m column with acetonitrile:water:0.5 triethylamine (85:10:5 v/v/v) as a mobile phase, at a flow rate of 1.0 ml/min, with detection at 240 nm. **Results:** This paper described the elaboration of simple and fast analytical procedure for assay of paracetamol, diclofenac sodium and omeprazole in tablets by RP-HPLC. The method was validated for the parameters like specificity, linearity, precision, accuracy. **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 described drugs in tablets.

Keywords: paracetamol, diclofenac sodium, omeprazole, RP-HPLC, validation

Introduction

The combination of analgesics of proven efficacy is a strategy intended to achieve one or more therapeutic goals, such as facilitating patient compliance, simplifying prescribing, improving efficacy without increasing adverse effects or decreasing adverse effects without loss of efficacy [1]. In certain cases, the co-administration of antinociceptive agents results in synergistic effects and the doses of the individual drugs can be substantially reduced [2]. Non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol (PAR) are drugs widely used to treat moderate to mild pain, but they are often inadequate against severe pain. Clinical studies on patients with musculoskeletal conditions, dental pain or postoperative pain have shown that combinations of paracetamol and NSAIDs may provide additive pain-relief [3]. Diclofenac sodium (DIC) is a NSAID with pronounced anti-rheumatic, anti-inflammatory, analgesic and anti-pyretic properties. Diclofenac inhibits prostaglandin synthesis, which play a major role in

the causation of inflammation, pain and fever. Paracetamol produces analgesia and antipyresis by a mechanism similar to that of salicylate, which involves inhibition of prostaglandin synthesis. However, it is generally known that NSAIDs are associated with gastrointestinal complications, whereas paracetamol is associated with very few side effects.

Omeprazole (OME), a proton pump inhibitor, inhibits acid secretion and provides protection against mucosal injury in patients receiving NSAIDs. It is well known that omeprazole was well tolerated and effective in preventing both gastric and duodenal ulcers in patients with arthritis receiving long-term NSAID therapy [4].

Certain successful attempts have been made for the simultaneous determination of diclofenac and omeprazole [5–8] as well as for assay of paracetamol and diclofenac in drug formulations [9–15]. Based on our current and ongoing referencing work, till date, we have not come across any reported HPLC method for simultaneous estimation of drugs

of interest in their combined dosage form. Present study emphasizes on the determination of paracetamol, diclofenac sodium and omeprazole in their model drug dosage forms by high performance liquid chromatography.

Experimental

Chemicals and reagents

Paracetamol, diclofenac sodium and omeprazole were supplied as standards. HPLC grade acetonitrile was used to prepare the mobile phase. All other chemicals applied for the chromatographic experiments were of a reagent grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C18, 250 mm x 4.6 mm, 5 μ m column eluted with a mixture of acetonitrile:water:0.5 triethylamine (85:10:5 v/v/v) as the mobile phase at flow rate of 1.0 ml/min. Detection was carried out by absorbance at 240 nm. The analysis was carried out at an ambient temperature and injection volume was 20 μ l.

Preparation of standard solutions

Reference solution (a): The solution was prepared by dissolving 25.0 mg of accurately weighed diclofenac sodium in methanol in a 100.0 ml volumetric flask ($C = 250 \mu\text{g/ml}$).

Reference solution (b): The solution was prepared by dissolving 10.0 mg of accurately weighed omeprazole in methanol in a 100.0 ml volumetric flask ($C = 100 \mu\text{g/ml}$).

Working reference solution: The solution was prepared by dissolving of accurately weighed 25.0 mg paracetamol in the

first step and in the second step by diluting 20.0 ml of reference solution (a) and 10.0 ml of reference solution (b) with methanol into a 50.0 ml volumetric flask. The concentrations of the investigated compounds in working reference solution were as follows: paracetamol – 500 $\mu\text{g/ml}$, diclofenac – 100 $\mu\text{g/ml}$ and omeprazole – 20 $\mu\text{g/ml}$, respectively.

Sample preparation

Twenty tablets were weighed and their average weight was calculated. These tablets were powdered and weight equivalent to half tablet containing 250 mg of paracetamol, 50 mg of diclofenac and 10 mg of omeprazole was taken in a 100 mL dilution flask. Then about 50 mL of methanol was added to it. The solution was sonicated for 10 min at an ambient temperature and diluted up to the mark with diluent, mixed well. The stock solution was filtered through a 0.45 μm Nylon syringe filter and 5.0 ml of the filtrate was diluted into a 25.0 ml volumetric flask to give a test solution containing 500.0 $\mu\text{g/ml}$ PAR, 100.0 $\mu\text{g/ml}$ DIC and 20.00 $\mu\text{g/ml}$ OME.

Results and discussion

The proposed method was validated as per ICH guidelines with respect to specificity, linearity, precision and accuracy.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs. The Fig. 1 showed typical chromatogram obtained from analysis of standard solution using the proposed method. The retention time observed – 2.41 min for paracetamol, 3.33 min for diclofenac sodium and 7.41 min for omeprazole permits a rapid assay, which is important for routine analysis.

Calibration and linearity

Calibration curves were plotted in the range of 125.0–1000.0 µg/ml for PAR, 25.00–200.0 µg/ml for DIC and 5.00–40.0 µg/ml for OME. The corresponding linear regression equations were $y = 185471.2x - 2653.2$ with a squared correlation coefficient R^2 0.9998 for PAR, $y = 28541.4x - 2341.9$ with a squared correlation coefficient R^2 0.9999 for DIC and $y = 56241.9x - 4412.2$ with R^2 of 0.9998 for OME, respectively. An excellent correlation existed between the peak areas and the concentrations of analytes.

Accuracy

Accuracy of the method was evaluated by standard addition technique, which was performed by addition of known amounts of pure PAR, DIC and OME to known concentrations of tablet powder and analysed by

proposed methods in triplicate. Results presented in Table 1 indicated good accuracy and showed no interference from tablet excipients.

Precision

Intraday precision (repeatability) was calculated using two concentrations of PAR (250, 750 µg/ml), DIC (50, 150 µg/ml) and OME (10, 30 µg/ml) in triplicate using proposed methods.

The inter day precision (reproducibility) was repeated three times on three different days for analysis of two different concentration (250:50:10, 750:150:30 µg/ml) for analyzed drugs. The values of % RSD (Table 2) for PAR, DIC and OME were found to be in the range from 0.34 to 0.86 indicating good repeatability and reproducibility of the analytical procedure.

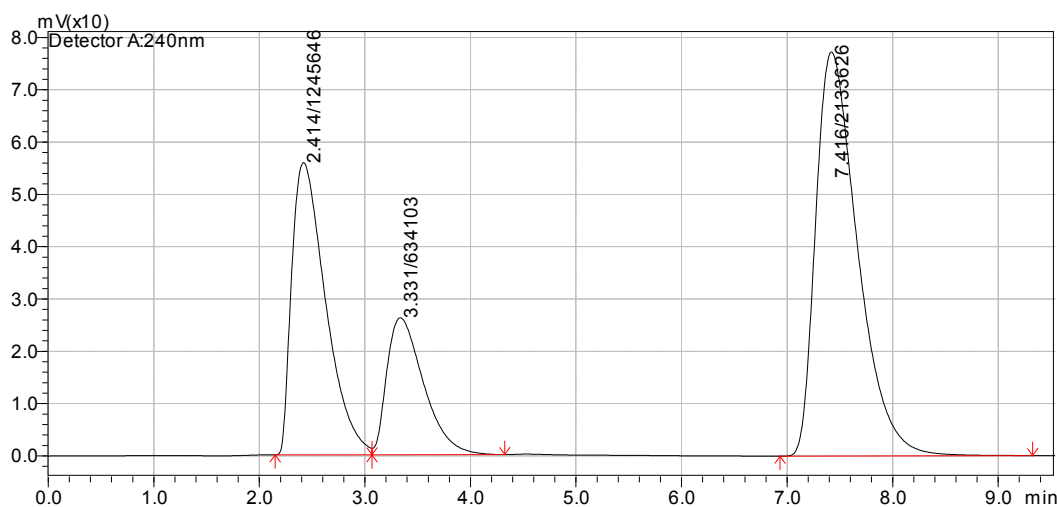


Figure 1. Chromatogram obtained from analysis of sample solution

Table 1. Recovery studies of PAR, DIC and OME

Drug	Amount taken (µg/ml)	Amount added (µg/ml)	Amount recovered ± SD (µg/ml)	% RSD
PAR	250.0	62.50	311.7 ± 0.92	0.31
		125.0	376.4 ± 1.27	0.35
		187.5	438.1 ± 1.16	0.26
DIC	50.00	12.50	62.17 ± 0.51	0.77
		25.00	75.25 ± 1.05	1.40
		37.50	86.91 ± 1.04	1.22
OME	10.00	2.50	12.46 ± 0.05	0.40
		5.00	15.01 ± 0.08	0.44
		7.50	17.49 ± 0.01	0.50

Table 2.

Precision	Amount taken (µg/ml)			% Mean *			% RSD		
	PAR	DIC	OME	PAR	DIC	OME	PAR	DIC	OME
Intra day	250	50	10	99.12	100.1	99.66	0.35	0.38	0.51
	750	150	30	100.4	100.0	100.4	0.61	0.65	0.63
Inter day	250	50	10	99.41	99.81	99.81	0.82	0.86	0.34
	750	150	30	99.69	99.58	99.62	0.72	0.85	0.47

*Mean of three determinations

Conclusion

The newly developed HPLC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation containing paracetamol, diclofenac sodium and omeprazole.

References

1. Raffa RB. Pharmacology of oral combination analgesics: rational therapy for pain. *Journal of Clinical and Pharmaceutical Therapy*. 2001; 26 (4): 257–264.
2. Fairbanks CA, Wilcox GL. Spinal antinociceptive synergism between morphine and clonidine persists in mice made acutely or chronically tolerant to morphine. *Journal of Pharmacology and Experimental Therapeutics*. 1999; 288 (3): 1107–1116.
3. Altman RD. A rationale for combining acetaminophen and NSAIDs for mild-to-moderate pain. *Clinical and Experimental Rheumatology*. 2004; 22 (1): 110–117.
4. Sharma P. Reliving 25 years of experience with omeprazole in acid-peptic diseases. *Journal of Association of the Physicians of India*. 2018; 66: 72-78.
5. Patel RK, Patel HR, Patel VA, Ganure AL, Patel LJ. Development and validation of RP-HPLC method for simultaneous determination of omeprazole and diclofenac sodium in capsule dosage forms. *Journal of Pharmaceutical Research*. 2012; 5: 1640-1642.
6. Paris W, Pajak A. TLC-Densitometry for determination of omeprazole in simple and combined pharmaceutical preparations. *Pharmaceuticals (Basel)*. 2022; 15 (8): 1016.
7. Chaitanya KS, Unnisa A. Novel UV-spectrophotometric method for simultaneous estimation of omeprazole and diclofenac in bulk and pharmaceutical preparations. *Journal of Pharmaceutical and Scientific Innovation*. 2014; 3: 428-433.
8. Wankhede SB, Borole VN, Chitange SS. Simultaneous spectrophotometric estimation of diclofenac sodium and omeprazole in capsules. *Asian Journal of Research in Chemistry*. 2011; 4: 1172-1175.

9. Phaneendra N. Quantitative analysis of paracetamol and diclofenac in combined dosage form by first derivative and simultaneous equation method in application to the determination of dissolution study. *International Journal of Pharmaceutical Science and Research*. 2012; 3 (10): 3783-3786.
10. Beeravolu S. Estimation and validation of UV-VIS spectrophotometric method for combined tablet dosage form of paracetamol and diclofenac sodium using extraction technique. *International Journal of Pharmaceutical Technology*. 2012; 4 (3): 4733-4740.
11. Sharma MC, Sharma S. Determination and validation of UV spectrophotometric method for estimation of paracetamol and diclofenac sodium in tablet dosage forms using hydrotropic solubilizing agents. *International Journal of PharmTech Research, coden (USA)*. 2011; 3 (1): 244-247.
12. Machmoud S, Amir M. Spectrum subtraction method for simultaneous determination of paracetamol and diclofenac sodium in their combined dosage forms. *Archives of Organic and Inorganic Chemical Sciences*. 2020; 4 (3): 523-528.
13. Emile B, Jeremie K, Issa I, Marguerite A, Elodie B, Justin K, Roland D. Transfer, validation and application of HPLC analytical method for simultaneous determination of paracetamol and diclofenac in tablets. *International Journal for Research in Biology and Pharmacy*. 2021; 7 (3): 1-5.
14. Dighe VV, Sane RT, menon SN, Tambe HN, Pillai S. Simultaneous determination of diclofenac sodium and paracetamol in a pharmaceutical preparation and in bulk drug powder by high performance thin layer chromatography. *Journal of Planar Chromatography*. 2006; 19 (112): 443-448.
15. Okoth OK, Yan K, Liu L, Zhang S. Simultaneous electrochemical determination of paracetamol and diclofenac sodium based on poly(diallyldimethylammonium chloride) functionalized graphene. *Electroanalysis*. 2015; 28 (11): 76-82.

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Specific marketing authorization procedures for early access to new medicines in the EU: a retrospective analysis (2015–2023)

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Introduction: The European Union (EU) pharmaceutical legislation establishes regulatory mechanisms aimed at facilitating a more flexible and accelerated scientific evaluation process for marketing authorization applications. Early access procedures are designed to expedite access to therapeutic areas with high unmet medical needs or lacking satisfactory treatment alternatives. **Aim:** This study aims to analyze the early access procedures within the EU in terms of quantity and types, and to categorize the medicinal products authorized under these procedures by therapeutic areas. **Materials and methods:** A retrospective analysis of the European Medicines Agency's (EMA) annual reports was conducted for the period 2015 to 2023. Early access procedures were categorized by number and type (conditional marketing authorization, approval under exceptional circumstances, and accelerated assessment). Medicinal products subject to these procedures were further classified by therapeutic areas. **Results:** In the years 2016 and 2017, the largest number of medicinal products underwent accelerated assessment procedures ($n = 7$ for both years). In 2021, the number of medicinal products receiving conditional marketing authorization remained consistent with that of 2020 ($n = 13$). This suggests a growing urgency due to the COVID-19 pandemic and an increased demand for expedited responses to unmet therapeutic needs. Approvals under exceptional circumstances peaked in 2020 and 2022 ($n = 5$ for each year). In 2023, eight medicines were recommended for conditional marketing authorization. Additionally, one medicine was authorized under exceptional circumstances, and three medicines received a recommendation for marketing authorization following accelerated assessment. The primary therapeutic areas in 2023 were oncology, hematology, and metabolism. **Conclusion:** Analysis indicates that medicinal products authorized for early access procedures between 2015 and 2023 predominantly belong to therapeutic areas such as neurology, hematology, endocrinology, oncology, and immunology, reflecting the expansion of these approaches into broader medical fields.

Keywords: EU, EMA, conditional marketing authorization, approval under exceptional circumstances, accelerated assessment

Introduction

All medicinal products for human use must be authorised before they can be placed on the market in the European Union (EU). The European system offers different routes for introducing a medicinal product to the European market [1]. In the EU there are two main routes for authorising medicines: a centralised procedure and a national procedure [2]. The centralized procedure allows the authorisation of medicines to the entire European Community market through a single application and assessment by the European Medicines Agency (EMA) [3]. The Agency's Committee for Medicinal Products for Human Use (CHMP) then carries out a scientific assessment of the application

and gives a recommendation to the European Commission on whether or not to grant a marketing authorisation [1]. Once granted by the European Commission, the centralised marketing authorisation is valid in all EU Member States as well as in the European Economic Area (EEA) countries Iceland, Liechtenstein, and Norway [2].

EMA listed the potential benefits from centralised procedure for EU citizens, which include [2]:

- A medicinal product is authorised in all EU member states at the same time;
- Centralised safety monitoring;
- Product information is available in all EU official languages ($n = 24$) at the same time.

Regarding its scope, centralised procedure is compulsory for the following medicinal products [2]:

- Containing **new active substance** and used in the treatment of HIV and AIDS; diabetes; cancer; neurodegenerative disorders; auto-immune diseases and other immune dysfunctions; viral diseases.
- Medicines derived from **biotechnology processes**, such as genetic engineering;
- **Advanced-therapy medicines** (e.g., gene-therapy, somatic cell-therapy, or tissue-engineered medicines).
- Orphan medicines (medicinal products used in the treatment of rare diseases).

When a medicinal product does not fall within the scope of the centralised procedure and if a pharmaceutical company wishes to obtain a marketing authorisation in several Member states, it can use a decentralised procedure or a mutual recognition procedure [1].

Specific mechanisms are laid down in the EU pharmaceutical legislation, which enable a more flexible and faster procedure for the scientific evaluation of applications for marketing authorization. The aim is to provide timely access to medicines in areas of unmet therapeutic needs, life-threatening conditions, or serious threats to public health [4, 5]. These specific procedures are established for medicinal products where the benefit of faster access exceeds the risk of having less comprehensive data. The main types of specific scientific assessment procedures that allow timely access to the EU market include conditional marketing authorisation (CMA), accelerated assessment (AA) and approval under exceptional circumstances.

Table 1 summarizes the main characteristics and differences between the EMA's specific pathways for marketing authorisation.

Table 1. Main characteristics of EMA's early access routes for marketing authorisation (*Created with BioRender.com*) [6, 7, 8]

Conditional marketing authorisation	Accelerated assessment	Marketing Authorisation under Exceptional circumstances
Ensuring timely access to medicines in areas of unmet medical need, life-threatening diseases, rare diseases, emergency situations.	Reserved for medicines that are of major public health interest and represent a therapeutic innovation	Comprehensive data on the efficacy and safety cannot be obtained.
Allows early approval on the basis of less complete clinical data than normally required		Medicines are subject to specific post-authorisation obligations and monitoring.
Authorisation is valid for 1 year and may be renewed annually.	Faster scientific assessment (within up to 150 days rather than up to 210 days).	Authorisation initially valid for 5 years (renewable)
Once the comprehensive data are provided, it can become a "standard" marketing authorisation		Reviewed annually to reassess the risk-benefit balance

Aim

This study aims to analyze the early access procedures within the EU in terms of quantity and type, and to categorize the medicinal products authorized under these procedures by therapeutic areas.

Materials and methods

A retrospective analysis of the European Medicines Agency's (EMA) annual reports was conducted for the period 2015–2023. Early access procedures were categorized by number and type (conditional marketing authorization, approval under exceptional circumstances, and accelerated assessment). Medicinal products subject to these procedures were further classified by therapeutic areas. Data on therapeutic indication, orphan status, and authorisation status were extracted from the European Public Assessment Reports (EPARs).

Results and discussion

In the years 2016 and 2017, the largest number of medicinal products underwent

AA procedures ($n = 7$ for both years) (Fig. 1). Under EU legislation, a medicinal product of major public health interest may be reviewed under an AA procedure [7]. This procedure is provided for by recital 33 and Article 14 (9) of Regulation (EC) No. 726/2004 [9].

In 2021, the number of medicinal products receiving CMA remained consistent with that of 2020 ($n = 13$). This suggests a growing urgency due to the COVID-19 pandemic and an increased demand for expedited responses to unmet therapeutic needs. The CMA tool was introduced for the first time in 2006. It has been recognised that CMA is an important tool for ensuring timely access to medicines in areas of unmet medical need [10].

Approvals under exceptional circumstances peaked in 2020 and 2022 ($n = 5$ for each year). In 2023, eight medicines were recommended for CMA. Additionally, one medicine was authorized under exceptional circumstances, and three medicines received a recommendation for marketing authorization following AA (Fig. 1).

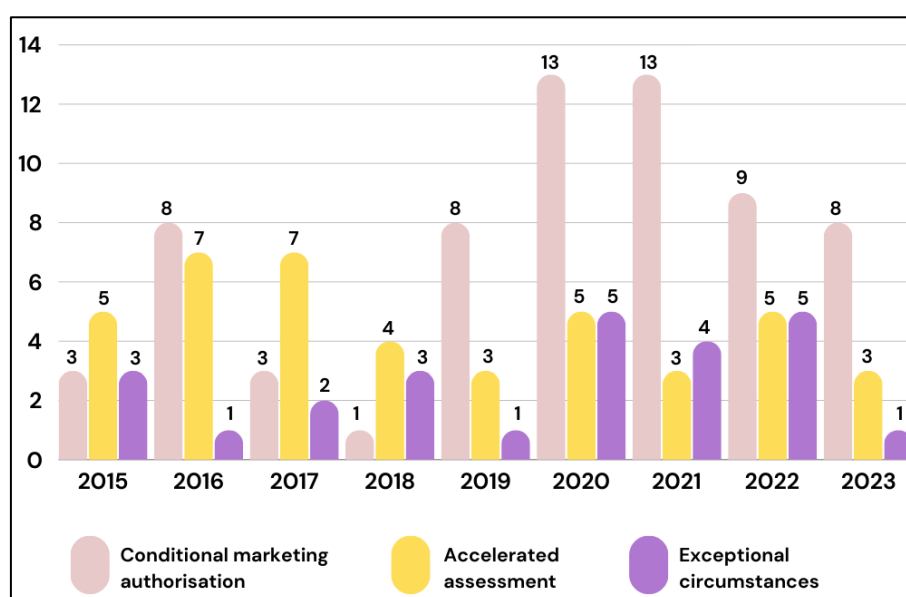


Figure 1. Medicines underwent an early access route for marketing authorisation (2015–2023). Created via Canva.com

A total of 66 CMAs have been granted in the period between 2015 and 2023. More than half of the CMAs ($n = 37$) were in the oncology area, followed by haematology, COVID-19, infection diseases, and neurology (Fig. 2). Oncology is also the primary therapeutic area for medicines recommended for approval under AA ($n = 15$). According to our search of the EMA database, just three cancer medications were authorised under exceptional circumstances during the analyzed period. The majority of medicinal products ($n = 9$) authorised under exceptional circumstances were in the metabolism/endocrinology area.

The primary therapeutic areas for medi-

cines that underwent an early access route for marketing authorisation in 2023 were oncology ($n = 8$), vaccines ($n = 2$), metabolism ($n = 1$), and haematology ($n = 1$) (Fig. 2). According to EPARs analysis, in 2023 there were five orphan medicinal products that were subject to early access procedures.

Conclusion

Analysis indicates that medicinal products authorized for early access procedures between 2015 and 2023 predominantly belong to therapeutic areas such as oncology, neurology, hematology, endocrinology, and immunology, reflecting the expansion of these approaches into broader medical fields.

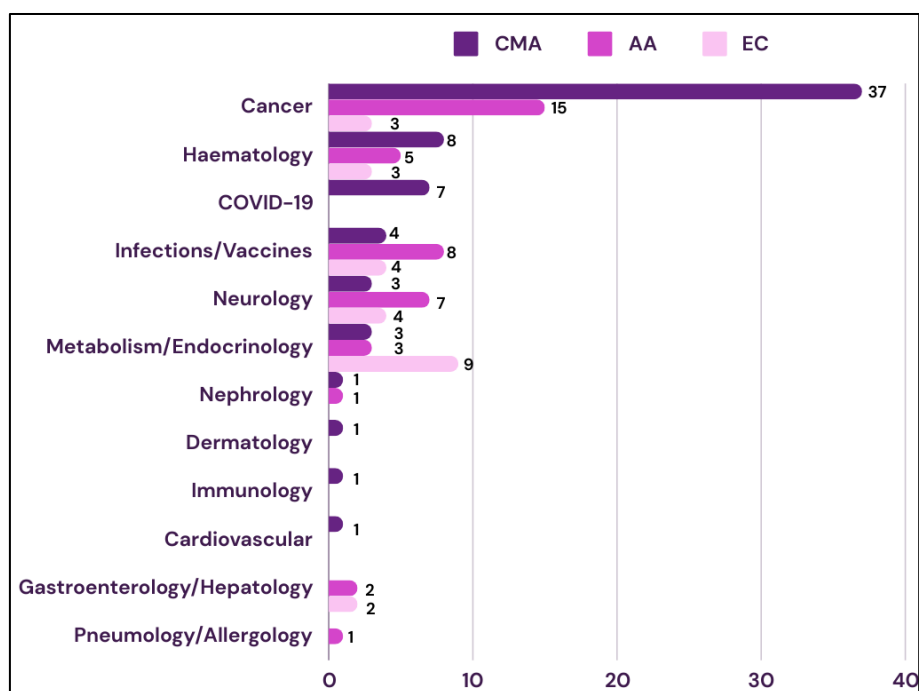


Figure 2. Early access marketing authorisations by the therapeutic area (2015–2023). Created via Canva.com

References

1. The European regulatory system for medicines: Bringing new safe and effective medicines to patients across the European Union https://www.ema.europa.eu/en/documents/leaflet/european-regulatory-system-medicines_en.pdf.
2. EMA: Authorisation of medicines. <https://www.ema.europa.eu/en/about-us/what-we-do/authorisation-medicines#from-lab-to-patient> (Assessed on 28 April 2024).
3. van der Vossen AC, Nachtnebel A, Wild C.

- Marketing authorisations under exceptional circumstances for oncology drugs: an analysis of approval and reimbursement decisions of four drugs. Ludwig Boltzmann Institut für Health Technology Assessment, Wien. 2013.
4. Getova-Kolarova V, Getov I. Marketing authorization procedures with negative opinion from EMA—tendencies and novelties in 2021–2022. *Annual for Hospital Pharmacy*. 2022;8(1):11–6.
5. Vallano A, Pontes C, Agustí A. The challenges of access to innovative medicines with limited evidence

in the European Union. *Front Pharmacol.* 2023;14:1215431.

6. European Medicines Agency pre-authorisation procedural advice for users of the centralised procedure https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/european-medicines-agency-pre-authorisation-procedural-advice-users-centralised-procedure_en.pdf.

7. EMA: Accelerated assessment. Available from: https://www.ema.europa.eu/en/documents/presentation/presentation-accelerated-assessment-aa-review-10-months-experience-new-aa-process-victoria-palmi_en.pdf

8. Ghadianian M, Schafheutle E. Comparison between European Medicines Agency and US Food and Drug Administration in Granting Accelerated Marketing Authorizations for Covid-19 Medicines and their Utilized Regulations. *Ther Innov Regul Sci.* 2024;58(1):79-113.

9. Regulation (EC) No 726/2004 of the European parliament and of the Council of 31 March 2004 <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:136:0001:0033:en:PDF>

10. Conditional marketing authorization, Available on: https://www.ema.europa.eu/en/documents/report/conditional-marketing-authorisation-report-ten-years-experience-european-medicines-agency_en.pdf.

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A comparative analysis of pharmaceutical care education in Bulgaria and France

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Introduction: Pharmaceutical care is now recognized as an indispensable aspect of pharmacy practice. To ensure pharmacists can effectively fulfill their primary mission, pharmacy students must acquire theoretical knowledge, practical skills, and competencies. Across many European countries, pharmaceutical care has been successfully integrated into pharmacy curricula for over two decades. **Aim:** This study aims to compare the pharmaceutical care courses at the Faculty of Pharmacy, Medical University of Plovdiv, Bulgaria, and the Faculty of Pharmacy, Aix-Marseille University, Marseille, France. **Material and methods:** The curricula of the faculties of pharmacy at the Medical University of Plovdiv, Bulgaria, and Aix-Marseille University, Marseille, France, were analyzed and compared. Our research focused on the pharmaceutical care syllabi, types of theoretical and practical classes offered, and teaching and evaluation methods employed. **Results:** Pharmaceutical Care is a compulsory course studied in the 8th semester of the fourth year of the pharmacy curriculum at the Faculty of Pharmacy, Medical University of Plovdiv. Teaching and evaluation methodologies include brainstorming, clinical case-solving, and roleplays. In France, there are two disciplines equivalent to Pharmaceutical Care. Teaching methods encompass problem-based learning, elearning, clinical case-solving, and self-training through viewing recorded cases. **Conclusion:** There are both similarities and differences in pharmaceutical care education between Bulgaria and France. While France offers two distinct classes for this discipline compared to one in Bulgaria, the pedagogical approaches vary. Nonetheless, both disciplines strive to provide accurate knowledge to pharmacy students.

Keywords: *Bulgaria, France, pharmaceutical care, pharmacy education*

Introduction

In the last few decades, the pharmaceutical sector worldwide has undergone a number of changes. In response to the need for change in the late 1980s, a new concept called “pharmaceutical care” appeared in the United States, based on the idea of modernizing the functions of pharmacists and their realization as health professionals in a market economy [1, 2]. Pharmaceutical care is a new professional direction for pharmacists that has been evolving for years. This concept does not aim to transform the role of clinicians or other healthcare providers but to meet the therapeutic needs of patients. Pharmaceutical care is the result of overprescribing of medicines, the presence of a wide variety of medicines on the pharmaceutical market, and the corresponding need for more information about them. This leads to a comprehensive study and coverage of drug therapy [2].

The need for pharmaceutical care is

supported by the fact that over 50% of prescription medicines are taken incorrectly [3]. Pharmaceutical care is a new pharmaceutical service for patients based on the cost-effectiveness approach that includes consulting a patient as a key element. The mission of practicing pharmacists today is to help patients achieve rational and safe use of their prescribed medicines. The theoretical training of pharmacists allows them to always know what information to give the patient. But to be convincing, to be able to provide appropriate feedback, to learn to listen effectively and to make the patient trust them, they must also acquire the necessary knowledge and skills for effective communication.

Since Strand and Hepler introduced the main definition of pharmaceutical care in 1990, pharmacy practice and education all over the world have undergone a transformation [4, 5]. In many European countries,

the concept of “pharmaceutical care” has been successfully applied in practice for more than 20 years [1, 2]. Nowadays, pharmaceutical care is considered an essential component of pharmacy practice. To ensure this primary mission of pharmacists can be accomplished, pharmacy students need to obtain theoretical knowledge, practical skills, and competencies [6].

There are five faculties of pharmacy in Bulgaria: 1) Faculty of Pharmacy, Medical University – Sofia; 2) Faculty of Chemistry and Pharmacy, Sofia University “St. Kliment Ohridski”; 3) Faculty of Pharmacy, Medical University – Plovdiv; 4) Faculty of Pharmacy, Medical University – Varna; 5) Faculty of Pharmacy, Medical University – Pleven (Figure 1).

The education in Bulgarian faculties of pharmacy is harmonized with all the requirements of the European Union (EU) and is comparable to the world’s leading

universities. The structure and content of the pharmacy curriculum for a pharmacy master’s degree in Bulgaria meet all the requirements of the relevant EU directives and current Bulgarian legislation [7]. According to the Unified State Requirements of Bulgaria, the curriculum for the Master of Science in Pharmacy degree includes a minimum of 3015 hours for the 26 compulsory subjects [7, 8]. One of these subjects is the course titled “Pharmaceutical Care”. This subject was introduced and offered in Bulgaria in 2000 as an elective course. In 2005, the course in pharmaceutical care changed its status from elective to compulsory [9, 10].

Aim

The aim of this study was to compare the pharmaceutical care education in the Faculty of Pharmacy, Medical University of Plovdiv, Bulgaria, and in the Faculty of Pharmacy, Aix-Marseille University, Marseille, France.

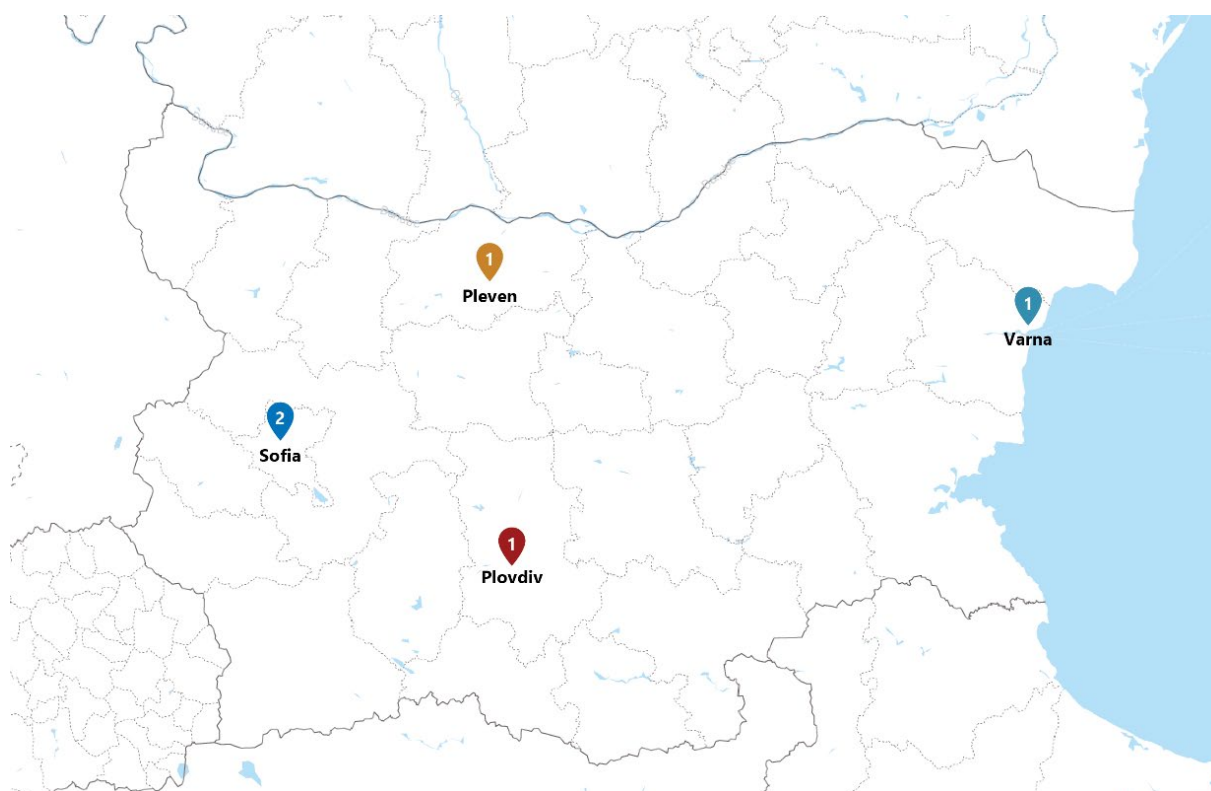


Figure 1. Google-based map of the pharmaceutical faculties in Bulgaria (numbers and location)

Material and methods

We carried out a content analysis of the legal and regulatory framework for pharmacy education and practice in the EU and Bulgaria. The following legal acts were analyzed: Directive 2013/55/EU; Law on the Professional Organization of Master Pharmacists in the Republic of Bulgaria; Bulgarian Law on Medicinal Products in Human Medicine; Decree on the Unified State Requirements for Acquiring Higher Education in a Master of Science in Pharmacy Degree.

The curricula of the faculties of pharmacy at the Medical University of Plovdiv, Bulgaria, and Aix-Marseille University, Marseille, France, have been studied, analyzed, and summarized. The scope of our research was the pharmaceutical care syllabus, the types of theoretical and practical classes included, and the teaching and evaluation methods employed.

Results and discussion

Pharmaceutical care education in Bulgaria (Faculty of Pharmacy, Medical University of Plovdiv)

The Faculty of Pharmacy at the Medical University of Plovdiv was established in 2003. Currently, the faculty has over 800 students. From the academic year 2016/2017, the faculty provided training to students in English. Education in the Faculty of Pharmacy is based on the quality of training, competitiveness, and cooperation [11]. The Erasmus+ exchange program provides a wide range of opportunities for student mobility for studies or for traineeships [11, 12]. The Faculty of Pharmacy actively participates in building and maintaining international relations with related institutions in Spain, France, Austria, Hungary, Italy, Latvia, North Macedonia, Belgium, Portugal, Romania, the Slovak Republic, Turkey, and the USA [11].

The discipline “Pharmaceutical Care” is one of the compulsory courses that is studied in the 8th semester of the fourth year of the pharmacy curriculum in the Faculty of Pharmacy at the Medical University of Plovdiv. The course “Pharmaceutical Care” includes two hours of lectures and two hours of practical classes per week, or a total of 60 hours per semester (30 hours of theoretical classes and 30 hours of training classes). These are the minimal number of hours for the subject “pharmaceutical care,” according to the Unified State Requirements for Acquiring Higher Education in a Master of Science in Pharmacy degree in Bulgaria [8]. In some of the faculties of pharmacy in Bulgaria, the course includes three hours of practical classes per week, or a total of 45 hours per week (e.g., at the Medical University of Sofia and Varna).

After completing the course of pharmaceutical care, students must have the following knowledge and skills:

- Knowledge about basic terminology, concepts, and models for verbal and non-verbal communication.
- Using different techniques to ensure effective communication.
- Skills for designing a pharmaceutical care plan.
- Assessing patient health literacy and confirming understanding.
- Skills for therapeutic behavior in common health problems requiring self-medication.
- Understanding the specific therapeutic needs of different groups of patients – children, patients with chronic diseases (diabetes, asthma, osteoporosis, cardiovascular diseases, oncologic diseases, etc.), pregnant and breastfeeding women, geriatric patients.
- Using different strategies and techniques for improving patient compliance and adherence.

- Identifying drug-related problems.
- Monitoring and follow-up of pharmacotherapy.
- Ensuring rational and safe medicine use.

The theoretical content of the course, teaching and evaluating methods used in the Faculty of Pharmacy at the Medical University of Plovdiv are presented in **Figure 2**.

Curriculum in French Pharmacy Programs (Faculty of Pharmacy, Aix-Marseille University, Marseille)

There are 24 individual faculties of pharmacy in France. The training takes place over 6 to 9 years, depending on the option chosen by the student. Pharmaceutical studies conclude with the passing of the State degree of Doctor of Pharmacy (PharmD), in French it is called “diplome d'état de Docteur en Pharmacie” [13].

The teachings are organized by pedagogical objectives in the form of teaching units (UE) including theoretical, methodological,

applied, practical and training courses [14]:

- The 1st cycle, sanctioned by the obtaining of the Diploma of General Training in Pharmaceutical Sciences (DFGSP) is obtained in 3 years.
- The 2nd cycle, sanctioned by the Diploma of Advanced Training in Pharmaceutical Sciences (DFASP) is obtained in 2 years.

The very early appearance of elective courses in the 3rd year allows students to develop their own professional project by customizing their course. The orientation towards one of the 3 fields (Officine, Industry, Internship) is made at the end of the first semester of the 4th year [14]. Pharmaceutical care is a discipline taught in the common core, for all students. The most similar courses to pharmaceutical care are taught during the 2nd semester of the 4th year. These classes are:

1. *Préparation à la prise de fonction hospitalière* = “Preparing to Become a hospital pharmacist” (50 hours, 6 ECTS)

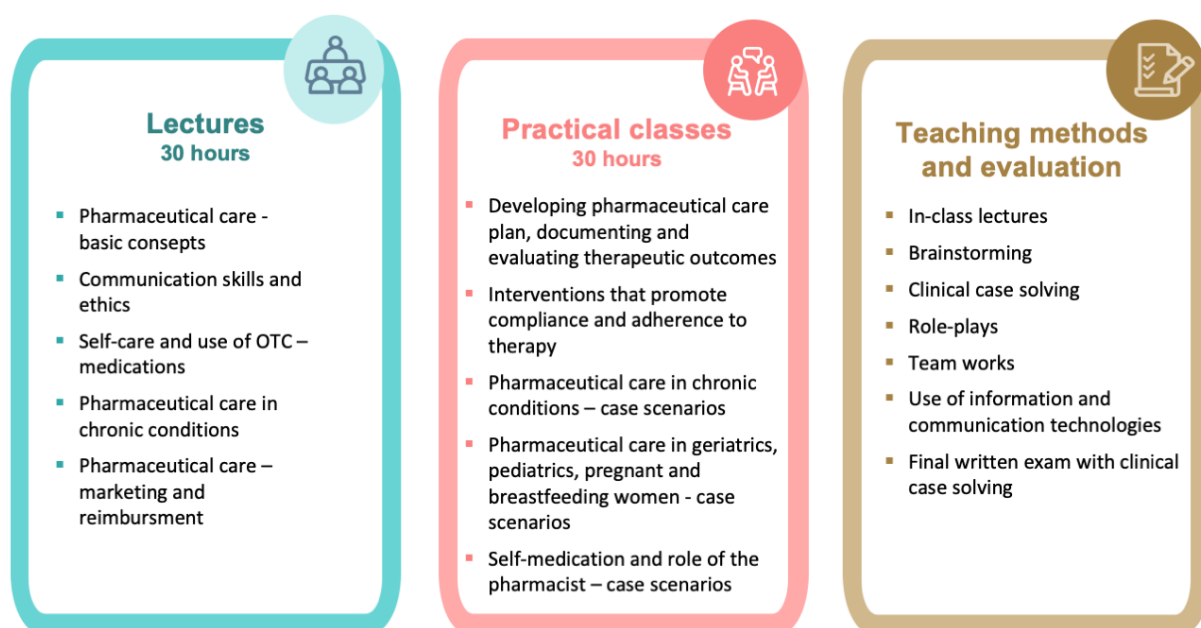


Figure 2. Teaching and evaluation methodology during pharmaceutical care course in Faculty of Pharmacy, Medical University of Plovdiv

2. *Le pharmacien et l'éducation thérapeutique du patient* = "The pharmacist and therapeutic patient education" (40 hours, 3 ECTS)

The first discipline consists of three different notions related to the Hospital sector:

1. Pharmaceutical Technical Skills, Quality and Good Practice
2. Clinical Pharmaceutical Skills
3. Biological skills

In the syllabus of the course "**Preparing to Become a hospital pharmacist**", there are some similar topics to those included in the pharmaceutical care syllabus in Bulgaria. These topics are:

- Clinical Pharmaceutical Expertise (Medication Review and The Personalized Pharmaceutical Plan)
 - Therapeutic follow-up
 - Medication reconciliation
 - Pharmaceutical interviews and therapeutic education of the patient
 - Biological diagnosis and monitoring of chronic diseases (e.g., diabetes, renal insufficiency, heart failure, etc.)

The second course ("**The pharmacist and therapeutic patient education**") has a lot of similarities with the pharmaceutical care course in Bulgaria, including the following topics:

- Educational Approach
- Educational Diagnosis or Shared Educational Assessment
- Evaluation and research in therapeutic patient education
 - Patient's expectations
 - Interactions with patients
 - Pharmaceutical interviews
 - Motivational interviewing
 - Interviewing patients with asthma
 - Interviewing patients on oral anticoagulant therapy
 - Interviewing patients with oncologic diseases

- Interviewing patients suffering from high blood pressure

The teaching methods include problem-based learning, e-learning, clinical-case solving, and self-training by viewing recorded cases [13].

Conclusion

In conclusion, we can summarize that there are some similarities and also some differences in pharmaceutical care education in Bulgaria and France. The management of this discipline is not exactly the same, but the content remains roughly similar. There are two different classes in France and one in Bulgaria for this discipline. The pedagogy is not the same, but both disciplines work to give accurate knowledge to pharmacy students.

References

1. Dimitrova Z, Petkova V, Gueorguiev S, Andreevska K, Staynova R, Grekova D, Madzharov V. Pharmaceutical care (3rd ed.). 2016; Tea Design Ltd., Sofia.
2. Petkova V. Pharmaceutical care – contemporary approach for patients' therapy optimization. Social Medicine. 2017; 12(2–3), 3–6.
3. Brown MT, Bussell JK. Medication Adherence: WHO Cares? Mayo Clinic Proceedings. 2011;86(4), 304–314. <https://doi.org/10.4065/mcp.2010.0575>.
4. Hepler CD, Strand LM. Opportunities and responsibilities in pharmaceutical care. American journal of hospital pharmacy. 1990;47(3):533-43. <https://doi.org/10.1093/ajhp/47.3.533>.
5. Martínez-Sánchez AM. Using the Delphi technique to determine objectives and topical outline for a pharmaceutical care course: an experience from the Cuban higher education system. BMC Medical Education. 2021;21:1-1. <https://doi.org/10.1186/s12909-021-02583-1>
6. Fresco P, Silva C. Pharmaceutical Care: a teaching experience. Pharmacy Education. 2011;11 (1): 190–193.
7. Getov I, Grigorov E, Lebanova H, Belcheva V. Comparative Analysis of The Structure and Content of Faculties of Pharmacy Curricula for Pharmacy Master Degree in Bulgaria. Social Medicine. 2017; 12 (2–3), 46–48.
8. Ministry of Education and Science, Republic of Bulgaria. (2009, April 7). Decree on the unified state requirements for acquiring higher education in Master of Science in Pharmacy degree. [In Bulgarian].

Lex.Bg. Retrieved March 10, 2022, from <https://www.lex.bg/laws/ldoc/2135501677>.

9. Petkova V, Dimitrova ZL, Radivoeva M. Implementation of pharmaceutical care knowledge in Bulgarian community pharmacies. *Pharmacy education*. 2006;6(2):107 – 110.

<https://doi.org/10.1080/15602210600625680>.

10. Petkova V, Atkinson J. Pharmacy practice and education in Bulgaria. *Pharmacy*. 2017;5(3):35. <https://doi.org/10.3390/pharmacy5030035>.

11. Faculty of Pharmacy – mission. Mu-Plovdiv.Bg. Retrieved April 20, 2022, from <https://mu-plovdiv.bg/en/faculties/faculty-of-pharmacy/mission/>.

12. Medical University of Plovdiv. Erasmus+ general information. Mu-Plovdiv.Bg. Retrieved April 20, 2022, from <https://mu-plovdiv.bg/en/erasmus/general-information/>.

13. Bourdon O, Ekland C, Brion F. Pharmacy education in France. *Am J Pharm Educ*. 2008;72(6):132. doi:10.5688/aj7206132

14. Faculty of Pharmacy of Marseille. Pharmacy studies | pharmacie.univ-amu.fr. www.Pharmacie.Univ-Amu.Fr. Retrieved May 10, 2022, from <https://pharmacie.univ-amu.fr/en/study-programs/pharmacy-studies>.

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IV. Therapeutic Session

Drug approach in Post-COVID high-grade ventricular arrhythmias: a case report

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A 50-year-old man who, 6 years ago, due to hypertrophic cardiomyopathy with obstruction and severe mitral regurgitation with normal coronary anatomy, underwent volume surgery-resection of the interventricular septum a modo Morrow and mitral valve replacement. Patient was taking as ambulatory treatment 2.5 mg Bisoprolol and Acenocumarol. 1 year ago, the patient experienced a moderate-severe COVID-19 viral infection. A few weeks later, an arrhythmia debuted – 8.447 ventricular extrasystoles – monomorphic, 114 ventricular couplets, 25 ventricular volleys, 3 episodes of ventricular tachycardia. Therapy was initiated with, Bisoprolol, Amiodarone without and with Bisoprolol, Mexitil – without effect. Due to resistance to the therapy, an MRI of the heart was performed with the aim of ablating the focus responsible for the ventricular arrhythmias – limited areas of fibrosis in the depth of the basal septum and in the lateral wall of the left ventricle subepicardial – of a non-ischemic type. In the desperate search at that time for a conservative solution, a nonselective beta blockers Propranolol was included with a gradual increase in the dose up to 40 mg t.i.d. The latter was able to abate the arrhythmias and alleviate the patients symptoms. Despite having a very narrow range of clinical application, Propranolol should be considered as a therapeutic option in cases of persistent arrhythmias.

Keywords: Arrhythmias, Propranolol, hypertrophic cardiomyopathy, COVID-19, ventricular tachycardia

Introduction

Our case pertains to the increasing number of COVID-19 associated cardiac complications. A 50 y.o. man presented to our practice with complaints of recurrent palpitations after having recovered from a COVID-19 infection. Magnetic resonance showed areas of subepicardial fibrosis, typical of infectious myocarditis. Holter ECG recording demonstrated a large number of ventricular extrasystoles (VES) and episodes of non-sustained ventricular tachycardia (VT) with a typical cool down phenomena before termination. Antiarrhythmic treatment with beta-selective beta-blockers (BB), Amiodarone and Mexiletine was ineffective at terminating the arrhythmia. Switching to a beta-non-selective BB Propranolol helped terminate the ectopic activity. The effectiveness of non-selective BB in treatment of persistent arrhythmias is a trait that has been underused in today's practice. Selective inhibition of

beta one receptors may be insufficient at suppressing some forms of arrhythmias that are due to automaticity.

Case presentation

A 50-year-old man who, 6 years ago, due to obstructive hypertrophic cardiomyopathy and severe mitral regurgitation with normal coronary anatomy, underwent volume surgery-resection of the interventricular septum a modo Morrow and mitral valve replacement. After having recovered from a COVID-19 infection patient complained of recurrent palpitations which severely lowered his quality of life. No syncope or reduced physical capacity were present. Patient denied ever having these types of complaints before. A 24 hours Holter ECG recording revealed a significant number of VES of around 10% and episodes of non-sustained VT. The latter was of a monomorphic type with a cool down phenomena right

before termination. Ambulatory treatment up to this point consisted of low dose Bisoprolol and Acenucomarol. Antiarrhythmic therapy was initiated. Firstly mono therapy with an increased dose of Bisoprolol was started but proved to be insufficient at terminating the palpitations. Addition of Amiodarone to the regiment proved to be futile as well. Amiodarone was switched with Mexiletine as to try and have a ventricle focused therapy but again effectiveness was suboptimal and the patient was still quite symptomatic with a developing anxiety associated with his constant palpitations. A last resort effort was made to try and conservatively abate the tachyarrhythmias with the use of a beta-nonselective BB Propranolol 40 mg t.i.d. The effect of treatment was prompt and the patient reported an almost complete cessation of his palpitations. The non-selective nature of Propranolol gives it a peculiar advantage in situations where inhibition of beta one receptors is insufficient. Both beta adrenergic receptors have sub cellular signalling cascades that promote and activate ion channels and storages leading to ion influx and overload. Blockage of both beta one and two receptors has an advantage as it allows complete inhibition of the signalling cascade at a sub cellular level, leading to a reduced activity of ion channels and reduced influx, thus reducing cellular excitability and automaticity.

Discussion and conclusion

Our case gives light to an almost forgotten antiarrhythmic drug, the use of which has been very limited and rare. Addition of Propranolol to the treatment regime of persistent arrhythmias is a proven strategy that need to find a more frequent use in the general practice.

References

1. Shin J, Johnson JA. Pharmacogenetics of beta-blockers. *Pharmacotherapy*. 2007 Jun;27(6):874-87.
2. Kalam MN, Rasool MF, Rehman AU, Ahmed N. Clinical Pharmacokinetics of Propranolol Hydrochloride: A Review. *Curr Drug Metab*. 2020;21(2):89-105.
3. Srinivasan AV. Propranolol: A 50-Year Historical Perspective. *Ann Indian Acad Neurol*. 2019 Jan-Mar;22(1):21-26.
4. Al-Kuraishy, H. M., Al-Gareeb, A. I., Mostafa-Hedeab, G., Kasozi, K. I., Zirintunda, G., Aslam, A., et al. (2021). Effects of β -blockers on the sympathetic and cytokines storms in covid-19. *Front. Immunol*. 12, 749291. doi:10.3389/fimmu.2021.749291
5. Bhatla, A., Mayer, M. M., Adusumalli, S., Hyman, M. C., Oh, E., Tierney, A., et al. (2020). COVID-19 and cardiac arrhythmias. *Heart rhythm*. 17, 1439–1444. doi:10.1016/j.hrthm.2020.06.016
6. Brodde, O. E. (1993). Beta-adrenoceptors in cardiac disease. *Pharmacol. Ther.* 60, 405–430. doi:10.1016/0163-7258(93)90030-h
7. Cuesta, A. M., Gallardo-Vara, E., Casado-Vela, J., Recio-Poveda, L., Botella, L.-M., and Albiñana, V. (2022). The role of propranolol as a repurposed drug in rare vascular diseases. *Int. J. Mol. Sci.* 23, 4217. doi:10.3390/ijms23084217
8. Gopinathannair, R., Merchant, F. M., Lakkireddy, D. R., Etheridge, S. P., Feigofsky, S., Han, J. K., et al. (2020). COVID-19 and cardiac arrhythmias: A global perspective on arrhythmia characteristics and management strategies. *J. Interv. Card. Electrophysiol.* 59, 329–336. doi:10.1007/s10840-020-00789-9
9. Grandi, E., and Ripplinger, C. M. (2019). Anti-arrhythmic mechanisms of beta blocker therapy. *Pharmacol. Res.* 146, 104274. doi:10.1016/J.PHRS.2019.104274
10. Grzešk, G., and Nowaczyk, A. (2021). Current modulation of guanylate cyclase pathway activity—mechanism and clinical implications. *Molecules* 26, 3418. doi:10.3390/MOLECULES26113418
11. Ibrahim, W. S., Ahmed, H. M. S., Mahmoud, A. A. A., Mahmoud, M. F., and Ibrahim, I. A. A. E.-H. (2021). Propranolol and low-dose isoproterenol ameliorate insulin resistance, enhance β -arrestin2 signaling, and reduce cardiac remodeling in high-fructose, high-fat diet-fed mice: Comparative study with metformin. *Life Sci.* 286, 120055. doi:10.1016/j.lfs.2021.120055
12. Li, W., Shenkar, R., Detter, M. R., Moore, T., Benavides, C., Lightle, R., et al. (2021). Propranolol inhibits cavernous vascular malformations by β 1 adrenergic receptor antagonism in animal models. *J. Clin. Invest.* 131, e144893. doi:10.1172/JCI144893
13. Lowe, M. D., Lynham, J. A., Grace, A. A., and Kaumann, A. J. (2002). Comparison of the affinity of beta-blockers for two states of the beta 1-adrenoceptor in ferret ventricular myocardium. *Br. J. Pharmacol.* 135, 451–461. doi:10.1038/sj.bjpp.0704450

14. Manolis, A. S., Manolis, A. A., Manolis, T. A., Apostolopoulos, E. J., Papatheou, D., and Melita, H. (2020). COVID-19 infection and cardiac arrhythmias. *Trends cardiovasc. Med.* 30, 451–460. doi:10.1016/j.tcm.2020.08.002
15. Sumpter, J. P., Runnalls, T. J., Donnachie, R. L., and Owen, S. F. (2021). A comprehensive aquatic risk assessment of the beta-blocker propranolol, based on the results of over 600 research papers. *Sci. Total Environ.* 793, 148617. doi:10.1016/j.scitotenv.2021.148617
16. Sofia Chatzidou , Christos Kontogiannis , Diamantis I Tsilimigras, Georgios Georgiopoulos , Marinos Kosmopoulos , Elektra Papadopoulou , Georgios Vasilopoulos , Stylianos Rokas (2018). Propranolol Versus Metoprolol for Treatment of Electrical Storm in Patients With Implantable Cardioverter-Defibrillator; DOI: 10.1016/j.jacc.2018.02.056
17. 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death; Authors/Task Force Members: Katja Zeppenfeld*† (Chairperson) (Netherlands), Jacob Tfelt-Hansen *† (Chairperson) (Denmark), Marta de Riva** (Task Force Coordinator) (Netherlands), Bo Gregers Winkel** (Task Force Coordinator) (Denmark), Elijah R. Behr (United Kingdom), Nico A. Blom1 (Netherlands), Philippe Charron (France), Domenico Corrado (Italy), Nikolaos Dagres (Germany), Christian de Chillou (France), Lars Eckardt (Germany), Tim Friede (Germany), Kristina H. Haugaa (Norway), Méléze Hocini (France), Pier D. Lambiase (United Kingdom), Eloi Marijon (France), Jose L. Merino (Spain), Petr Peichl (Czech Republic), Silvia G. Priori (Italy), Tobias Reichlin (Switzerland), Jeanette Schulz-Menger (Germany), Christian Sticherling (Switzerland), Stylianos Tzeis (Greece), Axel Verstraël (Belgium), Maurizio Volterrani (Italy), and ESC Scientific Document Group; *European Heart Journal* (2022) 43, 3997–4126 <https://doi.org/10.1093/eurheartj/ehac262>
18. Incessant ventricular tachycardia due to spontaneous automaticity in the Purkinje network inducing reversible left ventricular dysfunction; Eduardo Sosa, Mauricio Scanavacca; <https://doi.org/10.1093/europace/euq305> Published: 04 September 2010.
19. Utilizing the Cool-Down Phenomenon to Differentiate Supraventricular Tachycardias in a Patient with COVID-19 Infection; Khalid Sawalha, Vikram Baldini Gondhalekar, Nathan Klammer, Fuad Habash, and Hakan Paydak; Published online 2021 Aug 17. doi: 10.3390/clinpract11030068

Concomitant JAK-2 and BCR-ABL1 expression in a patient with chronic myeloid leukemia. Clinical evolution and cardiac impact

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Introduction: Double expression of Philadelphia chromosome and JAK-2 mutation in chronic myeloid leukemia (CML) patients is an uncommon condition. Little is known about the clinical evolution, best treatment modalities and the outcome of these patients. On the other hand JAK2V617F mutation alters endothelial function and promotes a large spectrum of cardiovascular complications. **Case presentation:** We present a case of a 63 years old male patient diagnosed with CML in 2017 with hyperleukocytosis, left shift, splenomegaly and Philadelphia chromosome transcripts. Standard therapy with Imatinib was initiated, but in 1-year suboptimal molecular response was registered. Therapy was switched to second – generation TKI-Nilotinib. In 2021 while in MMR 5.0 log, patient manifested headache and arterial hypertension. Lab tests showed significantly elevated Hb, Er, Hct and Plt while WBC and Differential formula were within normal ranges. Molecular tests proved JAK-2 mutation. Because of hypertension and arrhythmia, the patient was admitted in the Cardiology department. Arterial hypertension, ischemic heart disease, tachy-frequent atrial fibrillation, conduction disturbances, high grade aortic valve stenosis and multiple occlusions of peripheral vessels were found. TAVI was performed along with antihypertensive and anti-arrhythmogenic therapy. Nilotinib was replaced by Bosutinib to reduce cardiotoxicity. Currently the patient is still in MMR 5.0 log, but JAK-2 mutant allele burden is increasing, and the cardiovascular diseases are dominant.

Discussion and conclusion: There are no therapeutic standards for CML double positive for M-BCR-ABL and JAK-2. An unsolved problem is the impact of JAK-2 mutation on cardiovascular system.

Keywords: CML, JAK-2 mutation, cardiovascular complications

Introduction

Myeloproliferative neoplasms arise from hematopoietic stem cells with somatically altered tyrosine kinase signaling. Classification of myeloproliferative neoplasms is based on hematologic, histopathologic, and molecular characteristics including the presence of the BCR-ABL1 and JAK2 V617F. The pathognomonic marker of CML is Ph chromosome that results from a reciprocal chromosomal translocation between the ABL gene on chromosome 9 with BCR gene on chromosome 22 t (9;22). The resultant chimeric oncogene BCR-ABL displays autonomic tyrosine kinase (TYK) activity.¹ Their cellular effect is exerted through activation of multiple signal transduction pathways that transduce oncogenic signals. JAK-2 mutation is a somatic mutation, leading to activation of Janus kinase is a hallmark of the chronic myeloproliferative neoplasms (MPNs), found in Polycythemia vera (95%), essential thrombocythemia (60%), primary myelofibrosis (60%)^{2,3} Concomitant BCR-

ABL1 and JAK 2 mutations are considered to be mutually exclusive. Double expression of Philadelphia chromosome and JAK-2 mutation in chronic myeloid leukemia (CML) patients is an uncommon condition. Little is known about the clinical evolution, best treatment modalities and the outcome of these patients. On the other hand, myeloproliferative neoplasms (MPN) are chronic, clonal hematologic malignancies characterized by myeloproliferation and a high incidence of vascular complications (thrombotic and bleeding). Although MPN-specific driver mutations have been identified, the underlying events that culminate in these clinical manifestations require further clarification. What is known is that JAK2V617F mutation alters endothelial function and promotes a large spectrum of cardiovascular complications. We report a rare case of patient with CML, concomitant expression of JAK2V617F mutation and multiple cardiovascular complications which dominate the clinical presentation over the malignancies.

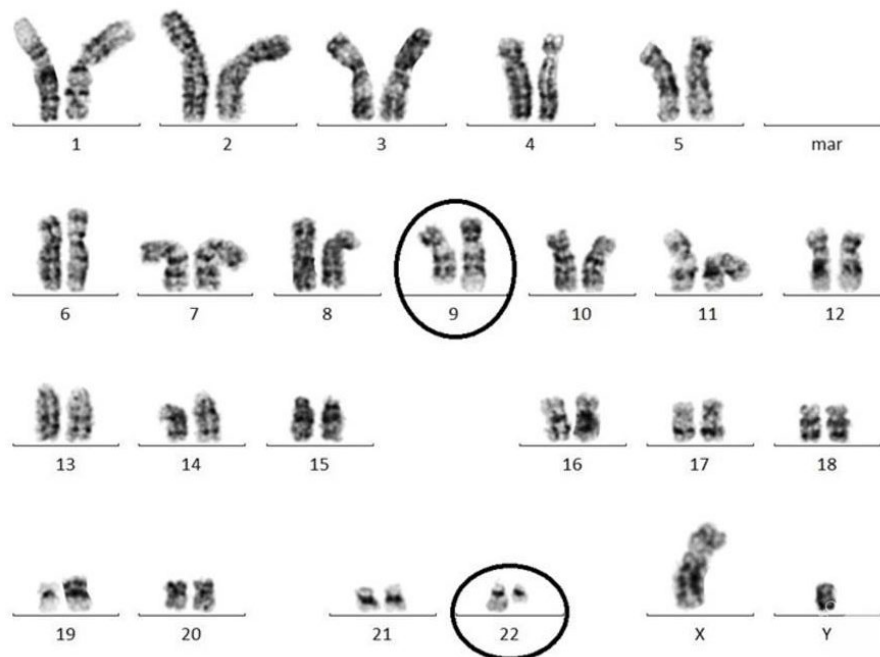


Figure 1. Caryogram showing Philadelphia chromosome t(9;22)

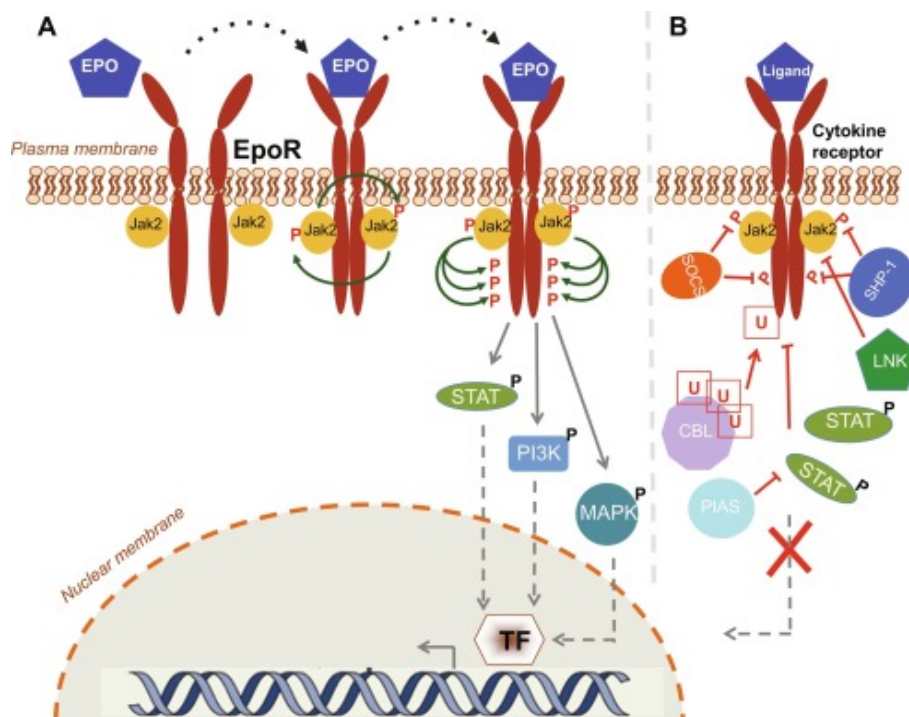


Figure 2. JAK2V617F mutation signaling

Case report

A 63-year-old male patient was diagnosed in 2017 with CML Ph⁺ and mBCR-ABL⁺ in late chronic phase. Risk group was calculated as high both according to Socal score 1.42 and Hasford score – 1318. The

diagnosis was made on a routine checkup. Physical examination revealed splenomegaly – 18/10 cm (abdominal ultrasonography). Laboratory findings: HGB 121 g/l, WBC – $220 \times 10^9/l$ (hyperleukocytosis), PLT – $459 \times 10^9/l$, Differential formula

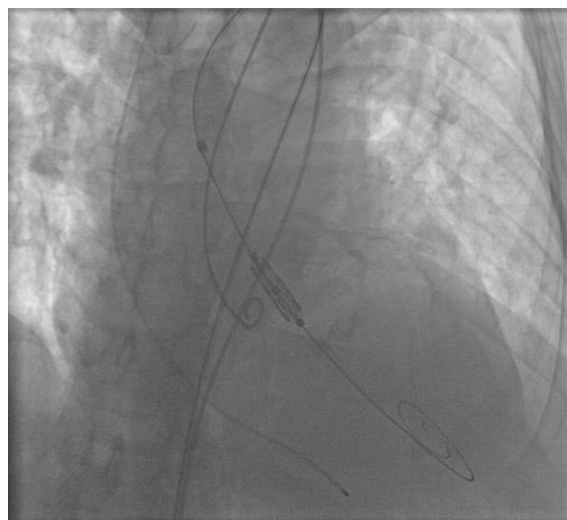
showed – MyBl 4%, ProMc 7%, Mc 26%, MtMc 8%, St 17%, Sg 30%, Eo 3%, Ba 3%, Mo 1%, Ly 3%. Myeloid hyperplasia was found in the myelogram Gr:Ery = 15:1 (predominantly ProMc, Mc, St and Sg). Initial therapy with Imatinib 400 mg per os was started. Therapy was switched to second – generation TKI – Nilotinib. At month 6 of therapy MR 5.0 (deep molecular response) was achieved, monitored by RT-PCR.

In 2021 patient started complaining from headache, high blood pressure with hypertensive crisis, itching of the lower extremities and reddish skin of the face. Laboratory findings showed erythrocytosis with elevated Hgb and Hct: Hgb 182 g/l, Ery – $6.37 \times 10^{12}/l$, Hct 0.58, WBC – $11 \times 10^9/l$, PLT $569 \times 10^9/l$, EPO – 2 mIU/ml (low), Blood gas analysis – normal. Molecular genetic testing proved JAK 2 p.V617F – (+), mBCR-ABL MMR5.0.

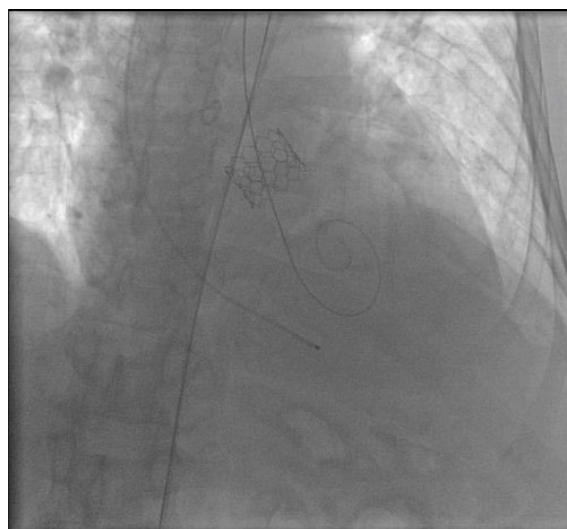
At the same time, the patient experienced astheno-asthenia, shortness of breath, palpitations, retrosternal pain at exertion. Due to these cardiac complaints, he was admitted to the Cardiology department. On physical examination – bilateral rhonchi in the base of the lungs were found, muffled heart sounds, systolic heart murmur, propagating towards the neck and carotid arteries, arrhythmia, pretibial oedema; ECG – atrial fibrillation, ventricular extrasystole 2nd Lown grade, left bundle branch block (LBBB); X-ray – cardiomegaly, with pulmonary congestion; Ultrasonographic examination of the heart: EF – 57%, dilated atriums and ventricles, high grade aortic valve stenosis – Pg mean (mean pressure gradient) – 43 mmHg, with fibrocalcinoses and low grade mitral and tricuspid insufficiency, pericardial effusion. Interventional coronarography proved double coronary artery disease, high grade aortic valve stenosis and peripheral arterial disease. Diagnosis included: Heart failure

3rd stage NYHA, dilated cardiomyopathy, high grade aortic valve stenosis, ischemic heart disease, two acute myocardial infarctions, peripheral artery disease, arterial hypertension 3rd grade, rhythm and conduction disorders – FA, ventricular extrasystoles, LBBB, CML and Polycythemia vera.

For cardiac complications therapy with Furanthril, Codaron, later replaced with Sotalhexal, Bisoprolol, Dapagliflozin, Trifas and Roteas was prescribed. Two stents in the proximal and distal segment of the LAD were placed Transcatheter aortic valve implantation (TAVI) was performed (Pictures 1 & 2).



Picture 1.



Picture 2.

For CML Nilotinib had to be replaced because of cardiotoxicity and Bosutinib 500 mg was started. Transient diarrhea as a side effect was registered. Erythrocytosis and high hematocrit are controlled by phlebotomies. Currently the patient is still in MMR 5.0 log, but JAK-2 mutant allele burden is increasing, and the cardiovascular disease is dominant.

Discussion

The presented case poses three main and unresolved in the literature so far problems:

1. The extremely rare combination of independent driver mutations – BCR-ABL and JAK 2 p.V617F (+).

2. Not sufficiently studied pathogenetic importance of mutation JAK2 on the vascular endothelium and its relationship with cardiovascular pathology.

3. Non available treatment guidelines.

BCR::ABL1 rearrangement and *JAK2/MPL/CALR* mutations were initially considered mutually exclusive genetic events. However, despite being a rare occurrence, a number of cases with concomitant Ph-positive and *JAK2*-mutated MPNs have been described with a frequency ranging from 0.2% to 2.5%, depending on different studies. The two mutations modulate the clinical expression of each other; In the suppression of one clone the mutational load of the other increases. In a systematic review, performed by Zanelli M et al in 2024 the majority of BCR:ABL1/JAK2 V617F double-positive cases fell into the group of Ph-negative MPN preceding CML (49.42%), followed by the group of CML and Ph-negative MPN occurring simultaneously (27.58%) and by the group of CML preceding Ph-negative MPN (22.98%). When Ph-negative MPN preceded CML, PV was the most commonly involved disease (20/43; 46.51%), followed by ET (12/43; 27.90%) and PMF

(8/43; 18.60%). On the other hand, when CML preceded Ph-negative MPN, the most frequently detected was MPN, NOS (7/20; 35%) followed by PMF (6/20; 30%), PV (5/20; 25%) and ET (2/20; 10%). When Ph-negative MPN and CML occurred simultaneously, PMF was the most frequent disease (10/24; 41.66%) followed by ET (6/24; 25%) and by MPN, NOS (3/24; 12.5%) and PV (3/24; 12.5%)⁷. The appearance of distinct symptoms, characteristic of given MPN, in the context of another, necessitates testing for a second mutation. Recent studies have found a link between MPN mutations and atherosclerosis and cardiovascular events (fig. 3). There is evidence that precursor endothelial cells carry JAK 2 – mutational load, which increases the risk of cardiovascular disorders⁸. The following mechanisms are discussed:

- JAK/STAT signaling pathway has a proven role in pro-inflammatory response.
- Greater adhesion of mononuclear cells to mutated EC.
- Increased surface expression of endothelial P-selectin and VWF, secondary to degranulation of Weibel-Palade bodies.
- Increased autocrine and paracrine secretion of VEGF – leading to microneoangiogenesis.
- Increased degree of arterial contraction in response of vasoconstrictors.
- JAK 2 mutation increases four times the risk of myocardial infarction; heart failure is more difficult to control; increases the risk of arterial thrombosis and shortened survival median in cardiovascular pathology.

Deterioration of cardiac function in our patient has complex pathogenesis – on one hand cardiotoxicity of Nilotinib and to lesser extent of Imatinib, on the other hand the above discussed effects of JAK2 mutation on endothelial cells on the basis of preexisting atherosclerosis.

Conclusion

JAK 2 mutated cells secrete inflammatory cytokines and growth factors that enhance bone marrow fibrosis, atherogenesis and thrombosis. In MPN there is dysfunction of blood cells, which impacts coagulation, both in the direction of thrombotic complications and in bleeding episodes⁹.

Mutational disorders in myeloproliferative diseases and cardiovascular pathology have a common intersection, which necessitates an appropriate therapeutic approach for both diseases. In the context of the

cardiovascular risk, TKI with the least cardiotoxicity (Bosutinib) is preferred. Other authors recommend Dasatinib as it covers a broad range of additional mutations along with mBCR-ABL¹⁰. There are publications stating that ACE-inhibitors improve the hematocrit and reduce the need for cytoreductive therapy. Priority is given to antiplatelet and anticoagulant therapy in MPN, which, however, may increase bleeding risk. Inhibition of the JAK-2 pathway by Ruxolitinib – reduces arterial and venous thrombosis.

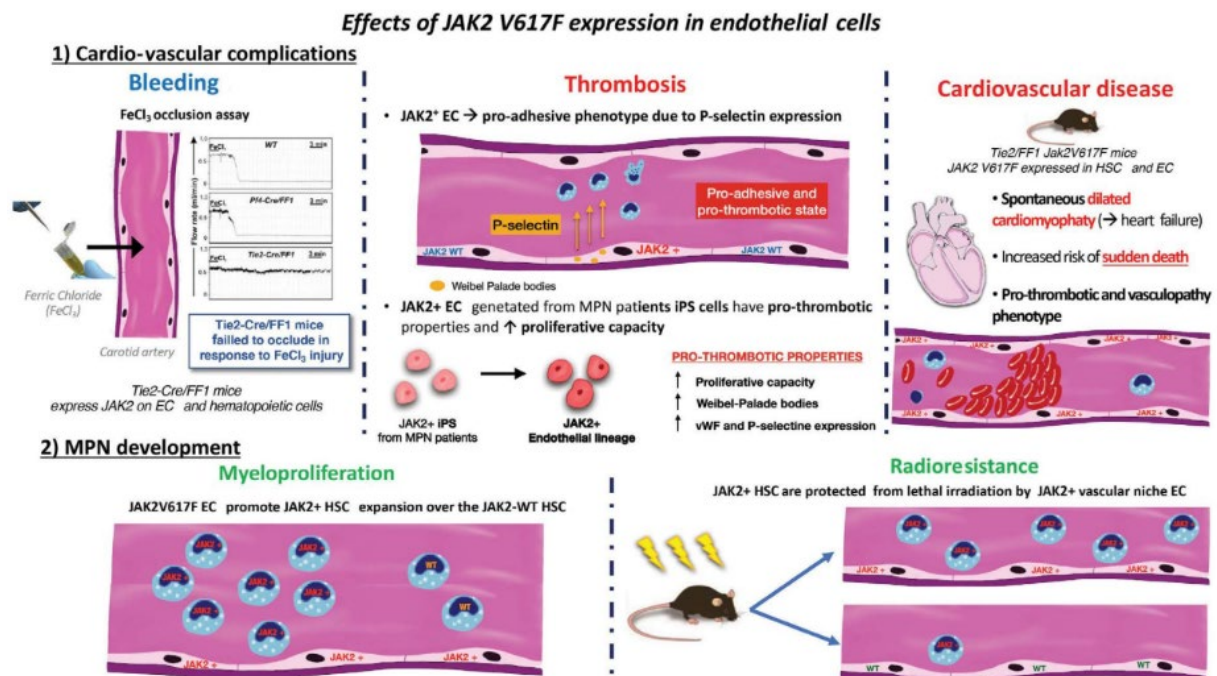


Figure 3. From: Farina M, Russo D, Hoffman R. The possible role of mutated endothelial cells in myeloproliferative neoplasms. *Haematologica*. 2021 Nov 1;106(11):2813-2823

References

1. Dai C, Chung IJ, Krantz SB. Increased erythropoiesis in polycythemia vera is associated with increased erythroid proliferation and increased phosphorylation of Akt(PKB)Exp Hematol. 2005;33(2):152-158
2. Levine RL, Wadleigh M, Cools J. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005;7:387-397
3. Steensma DP. JAK2 V617F in myeloid disorders: Molecular diagnostic techniques and their clinical utility. *J Molecular Diagnostics*. 2006;8:397-409
4. Pieri, L., Spolverini, A., Scappini, B., Occhini, U.,

- Birtolo, S., Bosi, A., et al. (2011). Concomitant occurrence of BCR-ABL and JAK2V617F mutation. *Blood* 118 (12), 3445–3446. doi:10.1182/blood-2011-07-365007
5. Martin-Cabrera, P., Haferlach, C., Kern, W., Schnitter, S., and Haferlach, T. (2017). BCR-ABL1-positive and JAK2 V617F-positive clone in 23 patients with both aberrations reveal biologic and clinical importance. *BJH* 176, 131–143. doi:10.1111/bjh.13932
6. Soderquist, C. R., Ewalt, M. D., Czuchlewski, D. R., Geyer, J. T., Rogers, H. J., His, E. D., et al. (2018). Myeloproliferative neoplasms with concurrent BCR-ABL1 translocation and JAK2V617F mutation: a multi-institutional study from the bone marrow

pathology group. *Mod. Pathol* 31 (5), 690–704. doi:10.1038/modpathol.2017.182

7. Zanelli, M., Bisagni, A., Sanguedolce, F., Broggi, G., Fragiasso, V., Zizzo, M., et al. (2024). Co-occurrence of JAK2-V617F mutation and BCR::ABL1 translocation in chronic myeloproliferative neoplasms: a potentially confounding genetic combination. *Front. Oncol.* 13, 1329298. doi:10.3389/fonc.2023.1329298

8. Farina M, Russo D, Hoffman R. The possible role of mutated endothelial cells in myeloproliferative neoplasms. *Haematologica*. 2021 Nov 1;106(11):2813-

2823. doi: 10.3324/haematol.2021.278499. PMID: 34320782; PMCID: PMC8561279.

9. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377(2):111-121.

10. Ali EAH, Al-Akiki S, Yassin MA. A Case Report of *BCR-ABL-JAK2*-Positive Chronic Myeloid Leukemia with Complete Hematological and Major Molecular Response to Dasatinib. *Case Rep Oncol*. 2021;14(1):690-694. doi: 10.1159/000514632. PMID: 34054462; PMCID: PMC8138239.

Left ventricular noncompaction cardiomyopathy in combination with atrial septal aneurysm and defect – better or worse?

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Introduction: Left ventricular noncompaction cardiomyopathy (LVNC) can occur in isolation or coexist with other cardiac and/or systemic anomalies. **Aim:** We describe the case of a woman who presented with heart failure for the first time at 42 years of age. **Material and methods:** The diagnosis was LVNC. Trans-thoracic echocardiography showed a trabeculated, sponge-like appearance of the ventricular apical and inferolateral segments as well as atrial septal aneurysm in combination with atrial septal defect (ASD) and left to right shunt. **Results and discussion:** After guideline based titrated medical management of heart failure, the patient was asymptomatic at follow-up examinations. The closure of the ASD was discussed. A decision for preserving the restrictive, left-to-right shunt as a natural method to reduce the degree of left atrial hypertension was made. We believe that left atrial decompression improves the atrioventricular interaction and global hemodynamic function. Reducing left atrial pressure and enlargement may be the answer why the incidence of atrial fibrillation is influenced, too. In addition, secondary pulmonary hypertension should be reduced by direct impact on the pulmonary congestion. In **conclusion** the case of LVNC in combination with atrial septal aneurysm and ASD is a model of natural hemodynamic compensation of heart failure.

Keywords: *noncompaction cardiomyopathy, atrial septal defect, treatment*

Introduction

Left ventricular noncompaction cardiomyopathy (LVNC) is a rare genetic disorder characterized by prominent endomyocardial trabeculations, deep intertrabecular recesses, and a thin compacted epicardial layer. LVNC is considered an unclassified type of cardiomyopathy, posing significant risks and perfect breeding ground for malignant arrhythmias, thromboembolic phenomenon, and left ventricular dilatation/dysfunction [1]. The exact pathogenetic mechanism through which LVNC develops remains incompletely understood. However, the most popular proposed theories suggest an arrest in intrauterine endomyocardial morphogenesis [2]. Despite its rarity and diverse presentation, LVNC often coexists with other cardiac anomalies, further complicating its diagnosis and management. The incidence of isolated LVNC ranges from 0.05% to 0.24%, with biventricular involvement occurring in 22% to 38% of cases. The prevalence of LVNC in combination with atrial septal defect (ASD) is around 8.3% [3]. The presence

of LVNC in combination with other disorders, such as atrial septal defect (ASD), was first documented in 1932, emphasizing the longstanding recognition of LVNC's complex presentations [4]. In this report, we present the case of a 42-year-old woman with LVNC and ASD, highlighting the challenges in diagnosis, management, and the potential role of ASD as a compensatory mechanism in mitigating the hemodynamic effects of LVNC. Through an exploration of the patient's clinical presentation, diagnostic criteria, and therapeutic considerations, we aim to contribute to the understanding and management of this complex cardiac condition.

Actual presentation of the case

Our patient, a 42-year-old woman, presented to the emergency room with complaints of fatigue, dyspnea, and tachycardia. She had no history of a previous chronic disease and no prior treatment. We immediately performed an electrocardiogram (ECG), which revealed sinus tachycardia, pathological progression of the R waves, and left

bundle branch block (LBBB). Imaging studies, including echocardiography, showed characteristic features of left ventricular noncompaction cardiomyopathy (LVNC), such as prominent trabeculations and intertrabecular recesses, along with hypomobility of the left ventricle. It also visualised the ASD. Further investigations, including ultrasound of the lungs and chest X-ray, demonstrated signs of congestion (B-lines/rocket lines on ultrasound; Kerley-B lines on chest x-ray) and cardiomegaly (enlarged left ventricle, diminishing the cardiothoracic ratio). Laboratory tests indicated elevated creatinine and reduced estimated glomerular filtration rate (eGFR), indicative of congestion. NT-proBNP was elevated, consistent with a heart failure presentation. Despite the typical clinical presentation, cardiac magnetic resonance (CMR), the gold standard for LVNC diagnosis, was not performed due to patient refusal. LVNC diagnosis relies on echocardiography, with specific diagnostic criteria compiled by various authors, including Chin, Jenni, and Stollberger. The natural course of LVNC involves an asymptomatic phase followed by symptomatic phases characterized by heart failure symptoms, arrhythmias (sudden cardiac death), and thromboembolic events (strokes and systemic embolisms). Treatment strategies include managing heart failure symptoms, thromboembolism prophylaxis (oral anticoagulants), antiarrhythmics, and potential consideration for heart transplantation. In our patient's case, the presence of LVNC in combination with an ASD introduced certain hemodynamic considerations. Upon follow up of the patient, we observed that the ASD served as a decompressing mechanism, dissipating some of the high systolic pressures arising from the LVNC. While our patient's mild ASD shunt provided a beneficial effect, ongoing follow-up is necessary to assess the

long-term impact of this combination on cardiac function and the potential need for interventional management.

Discussion

The case of our patient highlights the importance of recognizing the diverse clinical presentation of LVNC and the challenges associated with its diagnosis and management. LVNC often manifests with symptoms of heart failure and carries a poor prognosis due to the risk of arrhythmias, thromboembolic events, and progressive ventricular dysfunction. The combination of LVNC with other cardiac anomalies, such as an atrial septal defect (ASD) in our patient's case, further complicates management strategies and necessitates a multidisciplinary approach. Hemodynamic considerations in patients with concurrent LVNC and ASD involve understanding the interplay between these conditions and their impact on cardiac function. Shunt devices offer a method to decompress the heart by diverting blood flow, effectively reducing pressure on the left ventricle [5]. Traditionally, patients with LVNC are managed with lifelong diuretics and vasodilators, but these medications can have adverse effects, including the development of diuretic resistance. An alternative approach involves an interventional procedure where the atrial septum is punctured to induce an ASD, thus a shunt, aiming to relieve elevated pressures caused by LVNC. While this method is still under investigation, in cases where patients like ours already have an ASD, it provides a natural shunting effect, helping alleviate left ventricular pressure. However, if signs of right heart overload are detected, closing the ASD may be necessary. The future implications of combining ASD with LVNC remain uncertain, highlighting the need for further research to determine its efficacy and long-term effects. While our patient's mild ASD

shunt provided a beneficial effect in relieving some of the high systolic pressures associated with LVNC, long-term follow-up is essential to evaluate the efficacy of this combination as a potential therapeutic strategy.

Conclusion

In conclusion, LVNC remains a rare and challenging cardiomyopathy with significant morbidity and mortality. Early recognition, accurate diagnosis, and comprehensive management are crucial in improving outcomes for affected individuals. Our patient's case highlights the importance of considering LVNC in the differential diagnosis of patients presenting with heart failure symptoms, especially in the context of atypical ECG findings and characteristic imaging features. Further research is needed to better understand the pathophysiology of LVNC and explore innovative treatment modalities, particularly in cases of concurrent cardiac anomalies like ASD. A collaborative approach involving cardiologists, imaging specialists, and genetic counsellors is essential for optimizing the care of patients with LVNC and improving their long-term prognosis.

References

1. Srivastava S, Yavari M, Al-abcha A, Banga S, Abela G. Ventricular non-compaction review. *Heart Failure Reviews*. 2021 Jul 7
2. Nikolić A, Jovović L, Tomić S, Vuković M. Left ventricular noncompaction: clinical-echocardiographic study. *Vojnosanitetski Pregled* [Internet]. 2012 Jan 1
3. Marques LC, Liguori GR, Amarante Souza ACA, Aiello VD. Left Ventricular Noncompaction Is More Prevalent in Ventricular Septal Defect Than Other Congenital Heart Defects: A Morphological Study. *Journal of Cardiovascular Development and Disease*. 2020 Sep 25;7(4):39.
4. Yin L. Non-Compact Cardiomyopathy or Ventricular Non-Compact Syndrome? *Journal of Cardiovascular Ultrasound*. 2014;22(4):165.
5. Litwin SE, Borlaug BA, Komtebedde J, Shah SJ. Update on Atrial Shunt Therapy for Treatment of Heart Failure. *Structural heart*. 2022 Nov 1;6(6):100090–0.

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The impact of asbestosis in worker's health

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Introduction: We want to present a clinical case of a 76-year-old patient diagnosed with Asbestosis. More than 40 years ago he practiced a occupation related to asbestos exposure. The diagnosis was made 20 years after cessation of occupational contact with asbestos. The respiratory symptoms were triggered unexpectedly and cannot be linked to another lung disease. The progression of symptoms worsened the patient's quality of life. **Goal:** The aim is to raise awareness about the risk factors from the work environment. A purposefully taken occupational history could guide the diagnostic thinking of general practitioners towards the early diagnosis of this type of pneumoconiosis. **Materials and methods:** To describe this clinical case, the data from the patient's medical documentation and the results of functional and imaging lung tests were used. **Results and discussion:** Patients and doctors underestimate the importance of occupational history. They might not have information about the risk factors in the different occupations. The patient presented by us was in this situation. He didn't have information or suspected that the production activity he was performing was risk factor for his health. Exposure to asbestos, even after cessation can trigger a severe lung disease that significantly worsens the quality of life of the patients. **Conclusion:** Information about the occupational route and a targeted occupational history are of key importance for the diagnosis and prevention of dust lung diseases. They contribute to the early and accurate diagnosis of pneumoconiosis and can improve the quality of life of those affected.

Keywords: occupational disease, asbestosis, risk factors

Introduction

Asbestosis is a chronic pulmonary pathology and the result of exposure and inhalation of asbestos fibers, leading to interstitial fibrosis and impaired lung function. Asbestos encompasses a heterogenous group of natural, hydrated silicate mineral materials and was widely used in the past as one of the most popular construction materials due to its chemical and physical properties and inexpensive characteristics (1, 2, 3). Occupational risk groups include employees in the field of shipyard, construction, mining, aerospace engineering, but also electricians and railroad workers (4). Epidemiological studies indicate a higher prevalence in Asian countries, such as Japan, India and China, but also the UK (5). Production of Asbestos persists in developing countries, contributing to increased risk for development of late complications of Asbestosis, as Mesotheliomas, with a latency period of 15–40 years (6).

Actual presentation of the case

The subject of this report is a 76-year-old former engineer admitted to the Department of occupational diseases and clinical allergology ward, St. George university hospital, Plovdiv, with symptoms including productive cough and shortness of breath persisting for a few weeks. Chest Radiography revealed severe bilateral fibrosis and typical fibrotic plaques. A routine check-up on his hemogram showed significantly increased values in the laboratory analysis with elevated erythrocyte sedimentation rate, white blood cells, C-reactive protein and slightly low Hemoglobin values. Further investigations exposed restrictive ventilation disorder pattern on Spirometry and Oxygen saturation below 88%. The patient was physically examined and presented hyperresonant sound on percussion over the pulmonary area and prolonged expiration with wheezing on auscultation. He didn't know that he

was exposed to asbestos in his workplace and understood about his disease 30 years after the initial exposure. When his condition severely deteriorated and seek a medical evaluation, he understood he has Asbestosis.



Figure 1. Pulmonary emphysema with fibrous changes bilaterally basally & limited area bronchiectasis

The patient was treated with Methylprednisolone initially 40 mg 2 times per day intravenously, Salbutamol 3 times per day as an inhalation therapy, 2 times per day DuoResp Spiromax 160 mg/4.5 mg, Bromhexine 4 mg 2 times per day as an intramuscular injection. After initiation of treatment the patient showed improvement. The cough and dyspnea were reduced, and after 6 days of hospital care he was discharged without complaints. His SpO₂ improved up to 94%. He continues taking DuoResp Spiromax and is scheduled for routine checkups every 3 months to evaluate the status of a potential Mesothelioma development or exacerbation of his condition.

Discussion

The effects of asbestosis on workers' health are a crucial concern that has attracted considerable focus from medical professionals, health experts, policymakers, and society at large. In 2005 the EU banned asbestos.

Conclusion

This case highlights the significance of occupational history and anamnesis in diagnosing and preventing pneumoconiosis. Exposure to asbestos, even after cessation can trigger a severe lung disease that significantly worsens the quality of life of the patients. It is crucial to ensure Personal Protective Equipment, such as respirators and protective clothing, and it is essential to minimize direct exposure to asbestos fibers. Implementing Engineering Controls helps reduce airborne fiber levels. Administrative Controls, such as providing thorough training on asbestos hazards, and enforcing work procedures, play a vital role in preventing exposure. Regular Monitoring is also necessary aspects of prevention. Offering Medical Screening and Health Monitoring programs is crucial for early detection of asbestos-related diseases, enabling timely intervention and treatment. Public Awareness campaigns play a vital role in educating workers and the general public about asbestos risks (7).

References

1. AskMayoExpert. Asbestos-related pulmonary disease (adult). Mayo Clinic; 2020.
2. Lazarus AA, Philip A. Asbestosis. *Dis Mon.* 2011 Jan;57(1):14-26. [PubMed]
3. Virta RL. Mineral Commodity Profiles Asbestos; Circular 1255-KK; U.S. Geological Survey: Reston, VA, USA, 2005.
4. Agency for Toxic Substances and Disease Registry. Asbestos and your health. Available from: <https://www.atsdr.cdc.gov/asbestos/overview.html>. Accessed 2021 Oct 15.
5. Dodson RF, Hammar SP, editors. The history of extraction and uses of asbestos. In: *Asbestos: risk assessment, epidemiology and health effects*. Boca Raton, FL: CRC Press; 2006. p. 1-8.
6. Henderson DW, Leigh J. The history of asbestos utilization and recognition of asbestos-related diseases. In: Dodson RF, Hammar SP, editors. *Asbestos: risk assessment, epidemiology and health effects*. 2nd ed. Boca Raton, FL: CRC Press; 2011. p. 1-22.
7. World Health Organization. The WHO publishes reports and guidelines on occupational health and safety, including recommendations for preventing asbestos-related diseases. Available from: <https://www.who.int>.

A clinical case of Isolated Right sided infective endocarditis

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Introduction: Right-sided infective endocarditis (IE) accounts for 5% to 10% of all cases and is most commonly associated with intravenous drug use, intracardiac devices, and central venous catheters, which have become increasingly used in recent years. Isolated right-sided IE in the absence of the mentioned predisposing factors is extremely rare. **Objective:** To describe a clinical case of isolated right-sided IE in the absence of usual predisposing conditions. **Material and methods:** We present a 72-year-old man with a history of recurrent fever of 2–3 months, despite several courses of antibiotic treatment, with a typical inflammatory laboratory constellation. With suspicion of IE, he was admitted to the Cardiology Clinic for clarification. **Results:** Laboratory data revealed inflammation and anemia. X-ray showed evidence of bilateral pulmonary infiltrative changes and pleural effusions. Transthoracic echocardiography visualized a large and mobile vegetation of the tricuspid valve, with severe regurgitation. *Staphylococcus aureus* was isolated from blood cultures. A definite IE of the tricuspid valve was diagnosed. With an indication for early surgical treatment, the patient underwent surgery and prosthetics with a biological tricuspid valve prosthesis. Intraoperatively, a perimembranous ventricular septal defect was diagnosed and corrected. Post-operatively, another 30 days of antibiotic treatment was administered. **Discussion:** Clinical suspicion is key to making the diagnosis of IE and choosing the most appropriate therapeutic strategy. The presence of a congenital heart malformation with a left-to-right shunt explains the unusual isolated right-sided involvement. **Conclusion:** In cases of isolated right-sided IE, the possibility of congenital heart disease with left-right shunt should be considered.

Keywords: right-sided IE, congenital heart disease, diagnostics

Introduction

Infective endocarditis is an inflammation of endocardium of the heart valves, native or prosthetic, and in recent years also due to intracardiac devices and catheters. Right sided infective endocarditis (RSIE) accounts for 5% to 10% of all cases [1]. RSIE is most associated with intravenous drug use, intracardiac devices, and central venous catheters, which have become increasingly used in recent years. Isolated right sided IE in the absence of the above-mentioned predisposing factors is extremely rare. IE is a disease with a difficult diagnosis, which is often delayed due to the non-specific clinical picture. IE is a disease with a poor prognosis and in-hospital mortality at 16–25% [2]. The portal of entry of the infection and the potential causative agent is important for the selection of the initial empiric antibiotic treatment. Thus early surgical treatment is highly indicative to improve the survival rate in IE. In this

report we present a 72 year-old man that presented with right sided infective endocarditis with the presence of a ventricular septum defect (VSD). VSD is the second most common congenital heart abnormality seen in adults where there is an abnormal communication between the right and left ventricles and shunt formation consequently causing a mechanism of hemodynamic compromise [3]. By delving into the patients clinical presentation we will highlight the important diagnostic methods, investigations and therapeutic approaches that were conducted in order to attain the optimal outcome.

Presentation of clinical case

We present a 72 year-old man with a history of fever, fatigue, loss of appetite, cough, weight loss since July 2023. He had been treated with several courses of antibiotics, which only provided temporary relief. He had been hospitalized twice and was

consulted by various specialists across the board from a haematologist, gastroenterologist, urologist and pulmonologist. He had undergone dental procedures and cystoscopy two months prior to the clinical presentation of a fever. With suspicion of IE, he was admitted to the Cardiology Clinic for further investigative clarification in September, 2023.

His laboratory investigations on admission presented with white blood cell count of $15.7 \times 10^9/l$ (Normal range is 4.5 to $11.0 \times 10^9/l$); CRP of 208 mg/l (0 – 10 mg/l) and ESR of 94 mm/h (normal in a >50 year old male is $<20 \text{ mm/h}$) HGB – 93 g/l (Normal is 125 – 185 g/l); RBC – $3.6 \times 10^9/l$ (normal is 4.3 – $5.9 \times 10^9/l$); HCT – 0.29 L/L ; MCV – 81 fL (normal is 80 – 100 fL) [4]. These results suggested anaemia and chronic inflammation. To rule out myocardial infarction, we further examined creatine phosphokinase (CK), CK-MB and troponin levels, but found no cause for concern. Three blood cultures were performed, and all tested positive for *Staphylococcus aureus*.



Figure 1. Chest x-ray on initial presentation of the Patient

The chest x-Ray (figure 1) performed revealed a bilateral pulmonary infiltrative change with a predominance on the right side as well as a small bilateral pleural effusion.



Figure 2. Transthoracic Echocardiogram of the Patient

The transthoracic echocardiogram (TTE) (figure 2) revealed a large mobile vegetation on the lateral leaflet of the tricuspid valve, measuring $11/28 \text{ mm}$. The medial leaflet of the tricuspid valve also presented with a smaller vegetation. Furthermore, the patient additionally presented with a severe tricuspid regurgitation.

To validate our preliminary assessment of IE, we resorted to the well-established Dukes Criteria, following the guidelines set forth by the Duke-International Society for Cardiovascular Infectious Diseases Criteria for Infective Endocarditis [5] [6]. The diagnosis was based on two major criteria: positive blood culture and valvular vegetation. Following this diagnosis, the patient began a 4-week course of double intravenous antibiotic therapy, consisting of Vancomycin 2 g/day and Cefotaxime 2 g/day [7] [8].

Despite the therapy, the patient still exhibited large mobile vegetations, distal embolization in the lungs, and severe tricuspid regurgitation. These conditions necessitated early surgery. The patient underwent surgery for the implantation of a biological tricuspid valve prosthesis [9], leading to the detection and subsequent repair of a congenital perimembranous ventricular septum defect [10]. Postoperatively, the patient received another 30 days of antibiotic treatment.

To conclude, the case illustrates the critical importance of early and accurate diagnostics in managing IE. Selecting the correct therapeutic strategy is key to ensuring the most beneficial outcome for the patient. As in this case our patient presented with a right sided infectious endocarditis, a condition linked to the presence of a congenital heart malformation with a left-to-right shunt which adds further clarification to the unusual, isolated right-sided involvement. Therefore, in cases of isolated right sided IE the possibility of congenital heart disease with left-right shunt should be considered and actively diagnosed if applicable.

Bibliography

1. Lin, Cheng-Jei, et al. "Diabetes Mellitus: An Independent Risk Factor of In-Hospital Mortality in Patients with Infective Endocarditis in a New Era of Clinical Practice." *International Journal of Environmental Research and Public Health/International Journal of Environmental Research and Public Health*, vol. 16, no. 12, 25 June 2019, pp. 2248–2248.
2. Akinosoglou, Karolina, et al. "Native Valve Right Sided Infective Endocarditis." *European Journal of Internal Medicine*, vol. 24, no. 6, Sept. 2013, pp. 510–519.
3. Dakkak, Wael, and Tony I Oliver. "Ventricular Septal Defect." *Nih.gov, StatPearls Publishing*, 16 Jan. 2023.
4. Dean, Laura. "Blood Groups and Red Cell Antigens." *Www.ncbi.nlm.nih.gov*, 2005.
5. Fowler, Vance G., et al. "The 2023 Duke-ISCVID Criteria for Infective Endocarditis: Updating the Modified Duke Criteria." *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, vol. 77, no. 4, 4 May 2023, p. ciad271.
6. Baddour, Larry M, et al. "Infective Endocarditis in Adults: Diagnosis, Antimicrobial Therapy, and Management of Complications: A Scientific Statement for Healthcare Professionals from the American Heart Association." *Circulation*, vol. 132, no. 15, 2015, pp. 1435–86.
7. "UpToDate." *Www.uptodate.com, www.uptodate.com/contents/overview-of-management-of-infective-endocarditis-in-adults#*. Accessed 28 Apr. 2024.
8. Tackling, Gary, and Vasimahmed Lala. "Endocarditis Antibiotic Regimens." *PubMed, StatPearls Publishing*, 2020.
9. Pettersson, Gösta B., and Syed T. Hussain. "Current AATS Guidelines on Surgical Treatment of Infective Endocarditis." *Annals of Cardiothoracic Surgery*, vol. 8, no. 6, Nov. 2019, pp. 630–644.
10. "Heart Shunt: Types and Treatment." *Cleveland Clinic*.

Treatment strategy in a random HFrEF patient – it is always a challenge!

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Introduction: Treatment of heart failure with reduced ejection fraction (HFrEF) has been clearly defined in recent international guidelines. Official recommendations do not fully address every possible scenario in real clinical practice. **Aim:** We describe the case of a 58 years old male patient referred for a cardiology consultation before cataract surgery after an ECG. **Material and methods:** Transthoracic echocardiography showed a dilated, remodeled left ventricle, with segmental wall motion abnormalities and severe mitral regurgitation. Coronary angiography revealed chronic occlusion of right coronary artery and stenosis of circumflex artery. At that stage a referral for cardiac surgery was done and the patient asked for second opinion. Cardiac magnetic resonance was used for assessment of myocardial viability. **Results and discussion:** After heart team discussion a strategy for guideline based titrated medical management of heart failure was chosen. The patient was asymptomatic at follow-up examinations. The full spectrum of guideline recommended treatment was introduced. A two stage percutaneous revascularization was undertaken. After two months of follow-up the patient was in stable sinus rhythm, with improved left ventricular function and mitral regurgitation. **Conclusion:** In complex cases with secondary mitral regurgitation, decision-making isn't always straightforward. The Heart Team is essential to determine the best individualized management, starting from optimized pharmacological evidence based treatments.

Keywords: HFrEF, secondary mitral regurgitation, treatment

Introduction

HF is a multi-faceted clinical syndrome that impairs the heart's ability to pump blood efficiently, it is characterised by high levels of mortality and morbidity, decreased functional capacity and high rates of hospitalisations. HF affects more than 64 million people worldwide [1]. Therefore, it becomes global health priority to decrease the social and economic burden. Using evidence-based guidelines, it allows us to consider many factors that can lead to a diagnosis yet, despite the advancements, it is of the highest importance that treatment should be individualised due to the nature of the disease. Consequently, the management of heart failure requires a multidisciplinary approach to reduce total hospitalisations. This can be highlighted in the case of our patient whose diagnosis was incidental, which reveals how HF can remain unnoticed until a treatment algorithm is applied. This case report emphasises the treatment strategy deployed in a patient with heart failure, demonstrating how non-surgical methods aim to provide benefits on overall patient health without the associated

risks of surgical procedures. Through a detailed description of the patient's clinical presentation, diagnostic workup and therapeutic interventions, we aim to emphasise the importance of guide-line recommended therapy to optimise patient outcomes.

Our patient, 59 years old, was referred to the cardiology department after a routine ECG before his cataract surgery. Initially, he presented as asymptomatic but after thorough history taking and a physical exam it was discovered that he showed signs of fatigue and generalised weakness upon physical effort for the last 2–3 months, he was recently diagnosed with hypertension for the last 2 years and was undergoing treatment with ACE-inhibitors and calcium channel blockers. He revealed he was a smoker and his physical exam showed he had tachycardia, a gallop rhythm, and a systolic murmur. His laboratory investigations presented with an increased level of NT-proBNP and his metabolic panel demonstrated no causes for concerns besides a slightly elevated uric acid level. His ECG findings showed a left bundle branch block (LBBB) and atrial

fibrillation (AFib). In the next stage of his diagnostic workup, the echocardiograph showed us that he had left ventricular (LV) dysfunction with a decreased systolic function plus severe mitral regurgitation. To accompany the echocardiography, the patient underwent elective coronary angiography for etiological clarification of his diminished LV function. The coronary angiography revealed dual coronary artery disease with stenosis in the circumflex artery and total occlusion in the right coronary artery. This occlusion was causing basal dyskinesia and total hypokinesia paired with the LV dysfunction. After his initial workup, the patient was urged to continue his ACE inhibitors while also commencing beta-blockers (BB), a loop diuretic to relieve symptoms of congestion, and direct oral anticoagulants (DOACs) as prophylaxis due to his high risk of a thromboembolic event as determined by his high CHADsVAsc score. Using our patient as an example, we can understand the diagnostic algorithm for heart failure recommended by the recent ESC guideline [2]. Heart failure is initially suspected when a patient has identifiable risk factors, signs, or symptoms associated with heart failure and an abnormal ECG. For our patient, his hypertension diagnosis was a risk factor. His complaints of fatigue and decreased physical compliance and the findings of tachycardia, systolic murmur, and gallop rhythm fit the universal definition of HF (reference this document) – signs and symptoms. The abnormal ECG, as well as the presence of hypertension and elevated NT-proBNP, raised the clinical suspicion of HF. To confirm the diagnosis, the gold standard is performing an echocardiography, which showed LV dysfunction with a decreased ejection fraction (EF) of 33%. Using the phenotypic approach, we can classify the patient with HFrEF. After confirming the diagnosis determining the etiological cause is essential for commencing treatment. as it can

help us when it comes to commencing the treatment. So, for our patient, it was his coronary artery disease. In our patient's case, the underlying factor was his coronary artery disease.

Now, the question arises, what do we do next?

An option would be to refer the patient for an MRI or continue optimising the guideline-recommended therapy, we can also consider a valvular repair due to the severe mitral regurgitation or even implantable devices like ICD or CRT. cardioversion could also be an option in relation to rhythm control strategy. Following the therapeutic algorithm for Class I therapy [3], we absolutely recommended the patient to continue many of the medications he was already taking. Such as the ACE-I, BB, loop diuretics, and DOACs. Besides the already prescribed medication, the patient was started on MRA and SGLT2 inhibitor thus, completing the four pillars of recommended guideline therapy, that reduces mortality and morbidity in patients with HFrEF[4]. RAASi, BB, MRA and SGLT2 are indicated to reduce the mortality and risk of HF hospitalisations [5] [6] [7] [8].

One month later, during the follow-up with the patient, he reported feeling better yet he is still fatigued. His ECG shows sinus rhythm and his mitral regurgitation has reduced and his EF has improved yet it is still under 40%. His kidney function and potassium levels were closely monitored but there were no signs of concern. At this stage, the patient underwent an MRI which confirmed the findings from his echocardiography – LV dilation, a reduced EF, and a still present mitral regurgitation of first degree. An MRI was the preferred imaging modality as it allowed us to assess the viability of the myocardium. This would allow us to risk stratify if the patient would benefit from cardiac interventions and revascularisation. tThe MRI

findings revealed that there was preserved viability despite the occlusion of the right coronary artery so, the patient would benefit from interventions like revascularisation of the occluded right coronary artery. The patient had stents placed in the affected vessels – the circumflex artery and right coronary artery. At this point of his treatment, his ACE-I was swapped for ARNIs as per guideline recommendations [9] [10].

In using a holistic approach It is also important to highlight the importance of exercise rehabilitation in [11] in heart failure patients for improving exercise capacity, QOL and reduce HF hospitalization.

Conclusion

Asymptomatic LV dysfunction should be taken seriously in oligosymptomatic patients. Guideline-recommended pharmacotherapy can effectively modify secondary mitral regurgitation, with surgical correction being a possible consideration thereafter with the guidance of a heart failure specialist team. Heart failure specialist teams are essential in navigating treatment decisions towards optimising patient outcomes and stabilising the incidence of HF.

References

1. Savarese G, Becher PM, Lund LH, Seferovic P, Rosano GMC, Coats AJS. Global Burden of Heart failure: a Comprehensive and Updated Review of Epidemiology. *Cardiovascular Research* [Internet]. 2022 Feb 12;118(17). Available from: <https://pubmed.ncbi.nlm.nih.gov/35150240/>
2. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure. *European Heart Journal* [Internet]. 2021 Sep 21;42(36):3599–726. Available from: <https://academic.oup.com/eurheartj/article/42/36/3599/6358045?login=false>
3. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure. *European Heart Journal* [Internet]. 2021 Sep 21;42(36):3599–726. Available from: <https://academic.oup.com/eurheartj/article/42/36/3599/6358045?login=false>
4. Consensus trial study group. Effects of Enalapril on Mortality in Severe Congestive Heart Failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) [Internet]. *The New England journal of medicine*. 1987. Available from: <https://pubmed.ncbi.nlm.nih.gov/2883575/>
5. The SOLVD Investigators. Effect of Enalapril on Survival in Patients with Reduced Left Ventricular Ejection Fractions and Congestive Heart Failure. *New England Journal of Medicine*. 1991 Aug;325(5):293–302.
6. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, et al. The Effect of Spironolactone on Morbidity and Mortality in Patients with Severe Heart Failure. *New England Journal of Medicine* [Internet]. 1999 Sep 2;341(10):709–17. Available from: <https://www.nejm.org/doi/full/10.1056/NEJM199909023411001>
7. Zannad F, McMurray JJV, Krum H, van Veldhuisen DJ, Swedberg K, Shi H, et al. Eplerenone in Patients with Systolic Heart Failure and Mild Symptoms. *New England Journal of Medicine* [Internet]. 2011 Jan 6;364(1):11–21. Available from: www.nejm.org/doi/full/10.1056/NEJMoa1009492
8. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA, et al. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *New England Journal of Medicine*. 2019 Sep 19;381(21).
9. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, et al. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. *New England Journal of Medicine* [Internet]. 2020 Aug 28;383(15). Available from: www.nejm.org/doi/full/10.1056/NEJMoa2022190
10. McMurray JJV, Packer M, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin–Neprilysin Inhibition versus Enalapril in Heart Failure. *New England Journal of Medicine* [Internet]. 2014 Sep 11;371(11):993–1004. Available from: www.nejm.org/doi/full/10.1056/nejmoa1409077
11. Packer M, McMurray JJV, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, et al. Angiotensin Receptor Neprilysin Inhibition Compared With Enalapril on the Risk of Clinical Progression in Surviving Patients With Heart Failure. *Circulation* [Internet]. 2015 Jan 6 [cited 2019 Oct 28];131(1):54–61. Available from: <http://circ.ahajournals.org/content/131/1/54.long>
12. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure. *European Heart Journal* [Internet]. 2021 Sep 21;42(36):3599–726. Available from: <https://academic.oup.com/eurheartj/article/42/36/3599/6358045?login=false>

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Contained rupture of the thoracic aorta mimicking mediastinal malignancy: a case report

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The rupture of an aneurysm of the thoracic aorta (TAA) is a rare life-threatening emergency with a high mortality rate if the condition is not promptly diagnosed and treated. In a classic aortic rupture scenario the tearing or bursting of the aorta leads to massive internal bleeding, causing a sudden drop in blood pressure and rapid hemorrhagic shock. In this paper we present a rare case of a 71 year-old male patient with back pain and peripheral edema. Computed tomography (CT) examination was performed and a posterior mediastinal mass measuring 8.5 cm x 6.1 cm, that was compressing the esophagus, was found. The boundaries between the tumor and the aortic structures were hard to discern. The differential diagnosis of the finding included lymphoma, sarcoma or a primary esophageal tumor. Contrast-enhanced CT revealed a contained rupture of the thoracic aorta, limited by the surrounding structures and a thrombotic sheath, which explained the stable hemodynamics of our patient. This rare case underscores the importance of considering aortic pathology, specifically the possibility of a contained rupture, in patients with atypical mediastinal masses. We emphasize the role of contrast-enhanced CT and demonstrate that the awareness of this rare condition is crucial for accurate diagnosis and appropriate management.

Keywords: contained rupture, thoracic aorta, mediastinal mass, aortic pathology

Introduction

The thoracic aorta can be affected by a life-threatening condition known as a thoracic aortic aneurysm (TAA). This occurs when the aortic wall weakens, causing a dilatation of the vessel, which diameter can be dilated more than 50% (1).

Thoracic aortic aneurysms (TAA) unfold silently in about 95% of cases, often concealing their potential for catastrophic complications such as aortic dissection or rupture, resulting in substantial internal bleeding and subsequent hemorrhagic shock. Termed as “silent killers”, these aneurysms manifest predominantly in the root or ascending aorta, followed by the descending aorta and sporadically in the arch (1).

Our case shows an unusual presentation of a thoracic aortic aneurysm (TAA) in the descending part, ruptured but remained contained, having a tumor-like appearance on initial imaging. This case highlights the crucial need for using contrast-enhanced CT scans, as relying solely on a native CT scan could lead to misdiagnosing such aortic conditions.

Case presentation

71-year-old male presented to the emergency department with persistent high blood pressure despite optimized antihypertensive therapy, decreased urine output, periorbital and lower limb edema. The patient previously experienced back pain. His medical history included anterior and inferior myocardial infarction. In 2023 he was diagnosed with pyloric stenosis.

The performed CT scan of the thorax discovered a supradiaphragmatic solid formation (Fig. 1, white arrow) in the posterior mediastinum measuring 8.5 cm x 6.1 cm in the axial plane, with density on the non-contrast CT around 40–45 Hounsfield Units (HU), compressing the esophagus (Fig. 1, green arrow) ventrally. The mass appeared to infiltrate the thoracic aorta at the level of its distal part. No infiltration of adjacent bones or other structures was observed. Malignant etiologies that can affect the posterior mediastinum such as lymphoma, sarcoma or esophageal cancer were initially suspected. Laboratory results revealed

slightly elevated levels of urea and creatinine not allowing further evaluation using nonionic low-osmolality iodinated contrast media at this time. The patient did not have anemia, thrombocytopenia, or coagulation abnormalities. We were thus unable to reach definitive diagnosis by the native CT examination. Following hydration with isotonic saline intravenously, to minimize the risk of contrast-induced nephropathy, a contrast-enhanced CT scan was performed to assess the

relationship between the mediastinal mass and the thoracic aorta. What was found was a leakage of the contrast media through an anterior defect of the aortic wall. The leakage was bounded by thick walls up to 1.8 cm wide, which represented the thrombotic sheath, and which didn't enhance the same as the aortic lumen (Fig. 2, red arrow). Additionally the "draped aorta" sign was positive. All those CT findings led to the diagnosis of a contained aortic rupture.

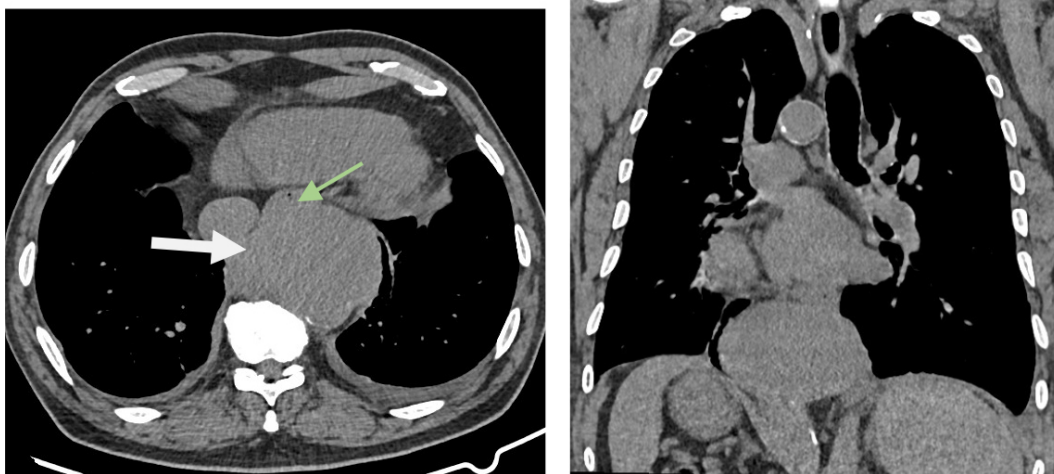


Figure 1. Native CT scan of the chest in axial and coronal plane demonstrating a solid retrocardiac formation measuring 8.5 cm x 6.1 cm, compressing the esophagus ventrally.

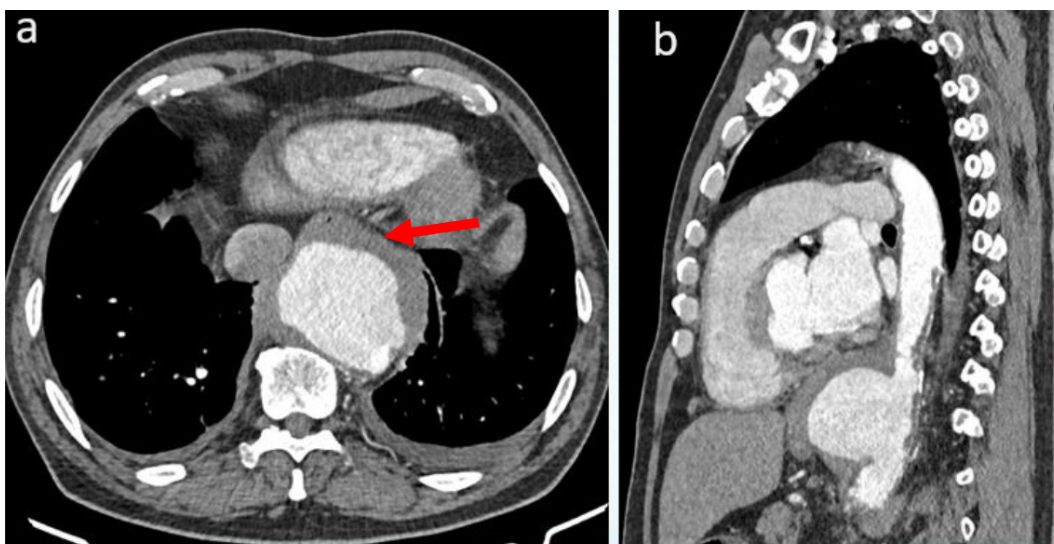


Figure 2. Contrast-enhanced CT-scan of the chest in axial (a) and sagittal (b) plane showing the contrast leakage, bounded by thick thrombotic wall, measuring up to 1.8 cm wide. Positive "draped aorta" sign with loss of fat planes between the aneurysm and the vertebral body.

Discussion

Chronic contained ruptures of the aorta, also known as sealed or contained leak, are indeed rare entities, first described by Szilagyi et al. and Galessiere et al. with less than 100 similar cases documented worldwide (2). Their distinct clinical presentation sets them apart from more common manifestations of thoracic aortic aneurysms (TAAs). While classic TAAs often present with acute symptoms such as severe back pain, dyspnea, dysphagia and hemodynamic instability, chronic contained ruptures, as seen in this case, can manifest with subtle or non-specific symptoms.

CTA (CT angiography) is the imaging modality of choice for diagnosis of aortic syndromes (3). Additionally, CT angiography incidentally detects aneurysms on exams performed for other purposes (4).

Acute aortic rupture appears on a contrast-enhanced CT as a discontinuity of the aortic wall with contrast media extravasation, and it's typically associated with a large, periaortic hematoma (5). In our case the formed hematoma had become contained and was limited by the surrounding structures by a thrombotic sheath, temporarily stabilizing the rupture. It is a life-saving phenomenon, buying time for diagnosis and treatment, because it acts like a barrier that contains the blood leaking from the ruptured vessel.

On CT images, draping of the aorta is highly indicative of deficiency of the aortic wall and a contained leak (6) (7). The "draped aorta" sign can be seen in contained aortic ruptures when the rupture site is posterior and sealed by the adjacent vertebral body. The posterior wall of the aorta moves to the anterior surface of the vertebrae, following which, normal fat planes between the aneurysm and vertebrae are lost (6) (7). Chronic contained rupture of an aortic

aneurysm may cause vertebral erosions in up to 30% of cases (6) (7). In our case the vertebral bodies were intact.

Although rare, similar cases of contained rupture of the thoracic aorta mimicking mediastinal masses have been reported in the literature. Chang et al., described a case of an 81-years-old man who presented with hemoptysis, and imaging revealed a mediastinal mass with peripheral increased FDG uptake on PET/CT, initially suspected to be malignancy (8). Likewise, Takahara et al., reported a case of a 70-year-old woman presented with back pain and a mediastinal mass on CT, initially diagnosed as lung cancer. However, the patient's rapid respiratory deterioration led to the diagnosis of a ruptured pseudoaneurysm of the thoracic aorta.

da Gama et al. reported a patient who was misdiagnosed as having an esophageal tumor causing his dysphagia and weight loss (10). In that case, the patient was diagnosed with a chronic contained rupture of a descending thoracic aneurysm by CT, and was surgically treated.

Conclusion

Chronic contained ruptures can appear similar to other mediastinal masses on imaging and the distinguishing is crucial for appropriate management. The true nature of the mass was confirmed by the contrast-enhanced CT scan that revealed a contained rupture of the thoracic aorta.

References

1. Faiza Z, Sharman T. Thoracic Aorta Aneurysm. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Updated 2023 May 1.
2. Szilagyi DE, Smith RF, Macksood AJ, Whitcomb JG. Expanding and ruptured abdominal aortic aneurysms. Problems of diagnosis and treatment. *Arch Surg*. 1961;83:395–408. doi: 10.1001/archsurg.1961.01300150069009.
3. Meng J, Mellnick VM, Monteiro S, Patlas MN. Acute Aortic Syndrome: Yield of Computed Tomography Angiography in Patients with Acute Chest Pain. *Canadian Association of Radiologists Journal*.

2019;70(1):23-28. doi:10.1016/j.carj.2018.10.011

4. Antunes BFF, Tachibana A, Mendes CA, Lembrança L, Silva MJ, Teivelis MP, Wolosker N. Signs of impending rupture in abdominal aortic and iliac artery aneurysms by computed tomography: Outcomes in 41 patients. *Clinics (Sao Paulo)*. 2021 Mar 8;76:e2455. doi: 10.6061/clinics/2021/e2455. PMID: 33681945; PMCID: PMC7920398.

5. Awal, Sandeep & Prasad, Niharika & Biswas, Som. (2022). CT evaluation of aortic dissection and other acute aortic syndromes: An update [Awal SS, Prasad N, Biswas S. CT evaluation of aortic dissection and other acute aortic syndromes: An Update. *Int J Radiol Radiat Ther*. 2022;9(5):159–165. DOI: 10.15406/ijrrt.2022.09.00343]. 9. 159-165. 10.15406/ijrrt.2022.09.00343.

6. Halliday KE, al-Kutoubi A. Draped aorta: CT sign of contained leak of aortic aneurysms. *Radiology*. 1996 Apr;199(1):41-3. doi: 10.1148/radiology.199.1.8633170. PMID: 8633170.

7. Hosn S, Lee S, Worsley C, et al. Draped aorta sign. Reference article, Radiopaedia.org. [Accessed on 15 Apr 2024].

8. Chang HS, Kim SJ, Kim YH. Contained Rupture of a Thoracic Aortic Aneurysm Mimicking a Malignant Tumor: a Case Report. *Nuclear Med Mol Imaging*. 2020 Oct 7;54(6):315-8.

9. Takahara Y, Nishiki K, Nakase K, Mizuno S. Ruptured pseudoaneurysm of the thoracic aorta mimicking lung cancer: A case report. *Thorac Cancer*. 2021 Mar;12(5):685-689. doi: 10.1111/1759-7714.13783. Epub 2021 Jan 9. PMID: 33421335; PMCID: PMC7919164.

10. da Gama AD, Martins C, Cabral G, Alves AG. Chronic contained rupture of a descending thoracic aortic aneurysm simulating an esophagus tumor. Conventional surgical management. *Rev Port Cir Cardiorac Vasc*. 2009;16:157–161

V. Somnology Session

The role of melatonin in hair loss

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Introduction: Until recently, melatonin has been considered a hormone regulating the circadian day-night rhythm. However, it modulates hair follicle growth and pigmentation. Anagen hair follicles are important sites of extrapineal melatonin synthesis. Melatonin is suggested to play a role in hair-cycle control and to down-regulate both apoptosis and estrogen receptor expression. **Aim:** To make a review of literature on the topic of the role of melatonin in hair loss. **Materials and methods:** We searched through PubMed and Google Scholar publications using the following keywords: “melatonin”, “hair loss”. A total of 54 publications published between 1988 and 2024 were reviewed. **Results and discussion:** A total of 9 studies were selected, with evidence of melatonin use in subjects with different types of hair loss. Most studies reported improved scalp hair growth, density, and hair shaft thickness among melatonin users. Topical melatonin dosage is 0.0033% or 0.1% solution/once-daily for and 1.5 mg twice-daily oral melatonin supplementation. It is suggested that melatonin plays a role in hair cycle regulation, because hair follicle is extrapineal melatonin source and peripheral melatonin target tissue. The intrafollicular melatonin synthesis and signaling is suspected to control endogenously hair growth by keratinocyte apoptosis modulation and by desensitizing the follicle to estrogen signaling. Melatonin may also counteract testosterone-induced cell actions. **Conclusion:** There is evidence to support melatonin use for hair growth, in people with hair loss conditions. Further studies should include more patients and investigate the mechanism of action.

Keywords: melatonin, hair loss, alopecia, androgenetic alopecia, trichology

Introduction

Melatonin is a pineal gland hormone, which plays a role in the bioregulation of sleep, circadian rhythms, pigmentation and cellular growth. It is also a very strong free radical scavenger, has cell-protective and immunomodulatory functions, and is considered an antiaging agent [1]. Anagen hair follicles (HF) are important sites of extrapineal melatonin synthesis and therefore the hormone is suggested to play a role in hair-cycle control [1–11].

Aim

The aim of this review of literature is to make a summary on the topic of the role of melatonin in hair loss.

Materials and methods

We searched through PubMed and Google Scholar publications using the following keywords: “melatonin”, “hair loss”.

A total of 34 publications published between 1988 and 2024 were reviewed.

Results

A total of nine studies were selected, with evidence of melatonin use in subjects with different types of hair loss. Most studies reported improved scalp hair growth, density, and hair shaft thickness among melatonin users. Topical melatonin solution dosage ranged from 0.0033% to 0.1% applied once-daily, while per oral treatment include 1.5 mg twice-daily supplementation. All studies suggested that melatonin plays a role in hair cycle regulation, because hair follicle is extrapineal melatonin source and peripheral melatonin target tissue. The intrafollicular melatonin synthesis and signaling is suspected to control endogenously hair growth by keratinocyte apoptosis modulation and by desensitizing the follicle to estrogen

signaling. Melatonin may also counteract testosterone-induced cell actions.

Discussion

Melatonin biology

Melatonin is famous for its chronobiotic characteristics, synchronizing and stabilizing the rhythms of the body. Melatonin is mainly synthesized by the pineal gland, but it is now known to be synthesized also by a large variety of extra pineal tissues [1, 2]. Other famous sites of its synthesis include lymphocytes, gastrointestinal tract, skin and HFs. It is also well known that hormone's synthesis and secretion is controlled by day and night rhythms, precisely by the presence of more or less light. More darkness increases its production and conversely, more light decreases it [2].

The hormone also has cytoprotective, immunomodulatory, and anti-apoptotic effects. It is considered one of the most powerful natural antioxidants, because it actions on free radicals and intracellular antioxidant enzyme system [3]. Melatonin has a highly lipophilic chemical structure, which allows for quick penetration of cell membranes and organelles. By doing so it protects intra- and extracellular components from oxidative stress [4]. It also has antitumor, hypopigmentation, anti-inflammatory, and immunomodulating activity. It maintains the integrity of the epidermal barrier and controls the thermoregulation of the body. Because of its beneficial effects on sleep, the hormone is used in the treatment of sleep disturbances, and it has a significant role in dermatoses with chronic pruritus like atopic dermatitis and chronic spontaneous urticaria. Data shows evidence for its role in photoprotection and skin aging and hyperpigmentary disorders (melasma) [3]. Melatonin has an important role in management of depression

and neurological conditions such as Alzheimer's, migraine and epilepsy, metabolic conditions including diabetes and obesity [2].

Melatonin and the hair follicle

Melatonin was considered to be secreted mainly by the pineal gland, but several important extrapineal sites of melatonin synthesis are now recognized. One of them is the anagen HF. In fact, the hormone has long been known to modulate hair growth and pigmentation. However, the mechanisms of action on the HF regarding growth control and pigmentation have not yet been completely understood [3]. HFs have a cycle, which includes a growth phase known as anagen, the regression phase known as catagen and cessation of the life cycle known as telogen. This cycle is stopped by the apoptosis of epithelial and melanocytic cells, which in turn loosen the hair shaft and move it in an upward direction. Another hypothesis for the important role of melatonin is that its levels decrease after the age of 20 and hair loss often start around that period [2].

Melatonin receptor expression in the hair follicle

It is suggested that some of the reported hair growth- and/or pigmentation- modulatory effects of the hormone are because of the direct effects of melatonin or of melatonin receptors expressed by HFs. Melatonin-like receptors in the keratinocytes and dermal papilla fibroblasts, were found to change at maximum in anagen and catagen HFs. [1]

Melatonin also controls hair cycle by downregulating apoptosis and estrogen receptor expression. Melatonin also interacts with androgen and estrogen receptor-mediated signaling pathways, exerting anti-androgenic effects [3]. Because of the regulation of the intrafollicular melatonin synthesis and signalling, the hormone may modulate

keratinocyte apoptosis during catagen and by desensitization of the HF to estrogen signaling [1]. The antiapoptotic effect of melatonin could be also related to the antioxidant effects of melatonin [1]. Because of melatonin is a free radical scavenger and DNA repair inducer, the active anagen hair bulb may also exploit melatonin synthesis in loco as a self-cytoprotective mechanism [3].

Studies on melatonin and (androgenetic) alopecia

Hair loss may affect negatively the self-esteem and quality of life of both man and women. The most common cause of hair loss in men and women is androgenetic alopecia (AGA), also known as male and female pattern hair loss. This is a genetically determined condition influenced by the effect of testosterone and dihydrotestosterone (DHT) on the HF and its life cycle. Only two therapeutic agents have been approved by the Federal Drug Administration (FDA) and European Medicines Agency (EMA) for treatment of AGA: oral finasteride (for men) and topical minoxidil (for women and men). Alternative active substances for the treatment of AGA are currently being investigated [6].

There is only one double-blind, randomized, placebo-controlled study that included 40 women (20–70 years of age) suffering from diffuse alopecia or AGA, which explored the connection between melatonin and hair loss. The study assessed whether topically applied 0.1% melatonin influenced the anagen and telogen hair rate. The solution was applied on the scalp once daily for 6 months. The assessment was done with trichograms while the monitoring of the treatment effects on the physiological melatonin levels were evaluated with blood samples. After the mentioned period, there was an increase in the anagen rate of 8.7%, while the placebo showed only an increase of

+3.89%. In women with diffuse alopecia, the increase of anagen rate was +1.6% [4, 5]. The study reported that the topical preparation with melatonin led to an increased anagen hair rate in occipital hair in the participants with female pattern hair loss compared with placebo. The diffuse alopecia group benefited from an increase of hair growth in the frontal hair line. Nevertheless, the occipital hair samples of patients with diffuse alopecia and the frontal hair counts of those with AGA also showed more anagen hairs. The study also reported that melatonin plasma levels were increased under the treatment, but did not exceed the physiological night peak [5]. This effect of the hormone might be interpreted as induction of hair growth by prolongation of the anagen phase due to the stop of the transition to catagen or by promotion of the transition from telogen to anagen [4]. In the study the diffuse alopecia was not classified under any of the non-scarring alopecias, so we cannot make conclusion about the type of hair loss that showed these results. Overall, the mode of action of the melatonin is not known, but the effect promises an induction of anagen phase [5].

Melatonin has been investigated as a candidate for an alternative to treat AGA, based on in vitro and in vivo studies that gave contradictory results. The first one reported stimulation of hair shaft elongation. The other one reported no effects of the hormone on human scalp hair growth or hair matrix proliferation in vitro [6].

Other five studies done consequently by the same research group, explored the effect of topically applied cosmetic preparation of melatonin content of 0.0033% used once daily for 90 days. The first study explored the pharmacodynamics of the applied drug and showed no significant influence on endogenous serum melatonin levels. The

analysis of the blood samples revealed that repeated application of the topical melatonin had no negative influence on the changes in physiological endogenous melatonin secretion. The maximum serum melatonin concentration in the melatonin group was higher than in the placebo group, and the mean serum melatonin concentration in the fertile women was considerably higher than in the postmenopausal women. No changes were detected in the vital parameters (blood pressure, heart rate) or the electrocardiogram within the treatment period [6]. Another observational study involved 30 men and women showed a significant reduction in the severity of alopecia after 90 days of treatment ($P < 0.001$) [6]. The third study, evaluated the efficacy of the topical melatonin preparation, which was applied to the scalp each night by 35 men (aged 18–41 years) with Stage I or II AGA (Hamilton/Norwood scale) for a period of 6 months. TrichoScan was used for measurement of hair count, hair density, hair diameter, anagen/telogen ratio, and vellus hair/terminal hair ratio. The results showed a statistically significant increase in the hair count and improved hair density of participants after 3 and 6 months. The increase in the hair count was 29.2% (3 months vs. 0 month) and 42.7% (6 months vs. 0 month); both values were statistically significant ($P < 0.001$). In the case of hair density an increase of 29.1% and 40.9% was determined after 3 and 6 months ($P < 0.001$). The “Comb test study” was an open-label observational study, which included 40 male and 20 female patients (mean age 41 years) with early-stage hair loss or hair-thinning, who applied the melatonin hair solution each evening for 90 days. Reduction in hair loss, was observed statistically significant among both women and men. In the last large, 3-month, multi-center study with more than 1800 patients, the percentage of people with

a 2- to 3-fold positive hair-pull test decreased from 61.6% to 7.8%, while the percentage of those with a negative hair-pull test increased from 12.2% to 61.5% ($P < 0.001$) [6]. These studies conclude that since safety and tolerability was good, the topical application of a cosmetic melatonin solution can be considered as a treatment option in androgenetic alopecia [6].

Another independent study also observed positive outcomes after topical melatonin use in subjects with AGA. They report improved scalp hair growth, density, and hair shaft thickness among melatonin users compared with controls. Effective topical melatonin dosage varies between 0.0033% or 0.1% solution applied once-daily for 90 to 180 days. The effective systemic dosage is 1.5 mg twice-daily oral melatonin supplementation for 180 days. There is evidence to support melatonin use to facilitate scalp hair growth, particularly in men with AGA [7, 8].

A study on vitamin C based nanovesicles, called aspasomes, that were loaded with melatonin, were found to be useful in the treatment of AGA. Melatonin aspasomes were topically applied and the treatment efficacy was assessed by hair pull test, histometric analysis and trichoscopy. Results showed clinically promising results including increased hair thickness, density and decreased hair shedding [9].

Another study explored melatonin carried in nanostructured lipids exhibiting high skin deposition in the treatment of AGA. The result of the treatment was assessed with hair pull test, histometric assessment, and trichoscopy. Results showed clinically desirable results with increased hair density and thickness and decreased hair loss [10].

Conclusion

Until quite recently, melatonin has been considered a hormone regulating the

circadian day-night rhythm and seasonal bi-orhythms. Recent investigations show that melatonin has multiple activities, including antioxidant and anti-inflammatory effects. Melatonin receptors are expressed in keratinocytes, melanocytes, and fibroblasts. On the scalp hair, melatonin promotes the anagen phase of the hair follicle. Melatonin may also have anti-apoptotic properties and can counteract testosterone-induced cell actions [7–11]. There is evidence to support melatonin use for hair growth, in people with hair loss conditions, and more specifically in AGA. Further studies should include more patients and investigate the mechanism of action of the hormone on the hair follicle.

References

1. Kobayashi H et al, A role of melatonin in neuroectodermal-mesodermal interactions: the hair follicle synthesizes melatonin and expresses functional melatonin receptors. *The FASEB Journal*. 2005; 19(12), 1710–1712. doi:10.1096/fj.04-2293fje
2. Singh M, Jadhav HR. Melatonin: functions and ligands. *Drug Discovery Today*, 2014, 19(9), 1410–1418. doi:10.1016/j.drudis.2014.04.014
3. Bešlić I et al, Melatonin in Dermatologic Allergic Diseases and Other Skin Conditions: Current Trends and Reports. *Int J Mol Sci*. 2023 Feb 17;24(4):4039. doi: 10.3390/ijms24044039. PMID: 36835450; PMCID: PMC9967801.
4. Fischer TW et al, Melatonin and the hair follicle. *J Pineal Res*. 2008 Jan; 44(1):1-15. doi: 10.1111/j.1600-079X.2007.00512.x. PMID: 18078443.
5. Fischer TW et al, Melatonin increases anagen hair rate in women with androgenetic alopecia or diffuse alopecia: results of a pilot randomized controlled trial. *Br J Dermatol*. 2004 Feb;150(2):341-5. doi: 10.1111/j.1365-2133.2004.05685.x. PMID: 14996107.
6. Fischer TW et al, Topical melatonin for treatment of androgenetic alopecia. *Int J Trichology*. 2012 Oct;4(4):236-45. doi: 10.4103/0974-7753.111199. PMID: 23766606; PMCID: PMC3681103.
7. Babadjouni A et al, Melatonin and the Human Hair Follicle. *J Drugs Dermatol*. 2023 Mar 1;22(3):260-264. doi: 10.36849/JDD.6921. PMID: 36877877.
8. Milani M, Fischer TW. Melatonin and hair physiology: reasons and clinical efficacy data as a topical treatment of androgenic alopecia. *Med Cutan Iber Lat Am*. 2014;42(1-3):30-36.
9. Hatem et al, Melatonin vitamin C-based nanovesicles for treatment of androgenic alopecia: Design, characterization and clinical appraisal, *Eur J Pharm Sci*, 2018, 122, 246–253
10. Hatem et al, Clinical Cosmeceutical Repurposing of Melatonin in Androgenic Alopecia Using Nanostructured Lipid Carriers Prepared with Antioxidant Oils, *Expert Opin Drug Deliv*, 2018, no. 10 (2018): 927–35. doi:10.1080/17425247.2018.1517740.
11. Sevilla A et al, Revisiting the role of melatonin in human melanocyte physiology: a skin context perspective. *J Pineal Res*. 2022; 72:e12790. doi:10.1111/jpi.12790

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Hypnic headache in patient with history of migraine attacks – a case report

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Introduction: The relatively new delineation of the hypnic headache as separate nosological entity combined with its low prevalence renders it mostly obscure and underrecognized by physicians. It is characterized by attacks occurring exclusively during sleep, leading to awakenings and subsequently reduced sleep quality. **Aim:** The aim of this report is to underscore the need for heightened clinical suspicion and comprehensive evaluation to differentiate hypnic headache from other primary and secondary headache disorders. **Materials and methods:** A 42-year-old woman, previously diagnosed with migraine, presented with complaints of multiple nighttime awakenings, accompanied by headache, at regular intervals of 90 minutes, nonrestorative sleep and snoring and witnessed apneas. She took up to 4 pills of non-steroid anti-inflammatory drug per night with limited effectiveness. A full unattended polysomnographic study (PSG) was performed. **Results and discussion:** The PSG data interpretation excluded the presence of sleep apnea. The patient-reported timing of the headaches coincided with REM episodes, which, added to their characteristics, were indicative of hypnic headache. The diagnosis was supported “ex juvantibus” by excellent response to caffeine treatment. Hypnic headache is not characterized by daytime episodes; therefore, we presume an overlap between hypnic headache and migraine in that case. **Conclusion:** The differential diagnosis of hypnic headache entails meticulous exclusion of secondary causes and consideration of overlapping features with other primary headache disorders. Despite the limited evidence-based guidelines for its treatment, the proposed therapeutic approach diverges from that of other types of headaches.

Keywords: hypnic headache, sleep, primary headache

Introduction

Hypnic headache (HH) is a recently discovered primary headache disorder characterized by headache attacks, occurring exclusively during sleep and leading to awakening. First described by Raskin in 1988¹, it has been continuously researched and in 2004 it was added to the International classification of headache disorders (ICHD). The differential diagnosis of HH necessitates the exclusion of secondary causes as well as other primary headache disorders². Here we present a clinical case of a patient with hypnic headache that was previously diagnosed with another type of primary headache – migraine.

Case presentation

A 42-year-old woman with a BMI of 25.6, who had previously been diagnosed with migraine, complained of multiple nighttime awakenings, caused by headache. They occurred 3–4 times a night at intervals

of around 90 minutes and lasted for 30–40 minutes, repeating for the better part of the month. The headache usually started with stomach discomfort, was either hemicranial or occipital and while previously accompanied by vomiting, more recently only the nausea persisted. For the pain she took Sibelium and multiple other medications, including triptans, with temporary effect on the frequency of the headache attacks. At the time of examination, she was taking up to 4 tablets Solpadein (acetaminophen/codeine/caffeine) per night. Her husband also noticed snoring and apneic episodes during sleep. She urinated 2–3 times during the night and did not report any sweating or dryness of the throat on awakening, but she complained of nonrestorative sleep. The headaches are more frequent during the summer. She reported no other concomitant illnesses.

The polysomnography (PSG) revealed impaired sleep microarchitecture with reduced slow-wave (N3) sleep – 16.4% of total

sleep time (TST) and reduced REM sleep – 14.5% of TST. Sleep efficiency was preserved – 91.2%. Apnea-hypopnea index (AHI) was 3.9/h, oxygen desaturation index (ODI) – 4.5/h and average desaturation drop was 3.2%.

Discussion

The most recent meta-analysis on the epidemiology of HH³ established the mean age of onset of HH to be 60.5 and with a slight prevalence in the female population. The pain is most often bilateral and of moderate or severe intensity³. It is a rare disorder with relative frequency of 0.21% among adults evaluated for headache while a population-based study in Iceland found no cases of HH and only 2 meeting the criteria for probable HH³. As per the third and last edition of the ICHD, the diagnostic criteria for HH are as follows:

A. Recurrent headache attacks fulfilling criteria B-E

B. Developing only during sleep, and causing wakening

C. Occurring on ≥ 10 days/month for > 3 months

D. Lasting from 15 minutes up to 4 hours after waking

E. No cranial autonomic symptoms or restlessness

F. Not better accounted for by another ICHD-3 diagnosis

The possibility that our patient's nocturnal headaches were migraine attacks was challenged after scrutinized analysis. However, her complaints and symptoms fulfilled ICHD-3's diagnostic criteria for HH². Furthermore, the attacks occurred exclusively during sleep and awoke the patient contrary to migraine attacks which happen during daytime as well and do not cause awakening⁴. The reported nausea, although typically associated with migraine, could accompany

an HH attack³. The duration of the headaches (up to 40 min) does not correspond to that of migraine attacks which last anywhere from 4 to 72 h⁴. It is also worth mentioning that the nocturnal headaches did not subside with the use of triptans and NSAIDS which are first line abortive treatment for acute migraine⁵. Interestingly, 25% of patients with HH have also been diagnosed with migraine³.

The normal respiratory indices from the PSG excluded the diagnosis of sleep-disordered breathing.

Cluster headache (CH) is another primary headache disorder to consider in the differential diagnosis of hypnic headache. Similarly to HH, the attacks usually arise from sleep and wake the patient, but there are often daytime attacks as well, the pain is more intense than HH and it is unilateral and accompanied by cranial autonomic symptoms (i.e., lacrimation, nasal congestion, ptosis) and restlessness which is uncommon for HH⁴. Another key characteristic of CH is that attacks cluster together in weeks long episodes and are followed by a remission lasting at least a month⁴. In contrast to HH, CH affects predominantly males with a ratio of 3:1.

Up until recently HH was considered a REM sleep-related disorder with 75% of recorded by PSG attacks occurring during REM⁶. This is what we also observed in our patient. However, increasing amounts of evidence point to independence of any particular sleep stage⁷. It is, nevertheless, considered a chronobiological disorder since it most often occurs at the same time every night – also called “alarm clock headache.” For 51% of the diagnosed this is between 2 and 4 a.m.⁸. This observation has given rise to a proposed pathophysiologic mechanisms of the disorder, linking it to an impairment of the function of the suprachiasmatic

nucleus (SCN). Its most well-known role is the generation of circadian rhythmicity in physiological functions, one of which – sleep, is influenced by the control of the SCN on melatonin secretion. Furthermore, the SCN has afferent and efferent projections to the periaqueductal grey – a key antinociceptive structure⁹. The epidemiological findings of the mean age of onset being around 60 could explain an age-related impairment in the SCN leading to inadequate melatonin secretion which is evident in the decrease of slow wave sleep and the increase in daytime naps and nighttime awakenings in aged people⁶. In more recent studies, there has been a shift of attention to the posterior hypothalamus's involvement in the pathophysiology of HH, with evidence for grey matter volume loss in this area in patients suffering from HH¹⁰. The posterior hypothalamus has influence both on sleep regulation and on nociception due to its anatomical connections to the nuclei of the ascending activating system¹¹ and to structures of the descending control of pain perception such as the periaqueductal grey and locus coeruleus¹⁰.

The chronobiological nature of HH is further evidenced by its successful treatment with lithium. Lithium increases serotonin release and decreases serotonin receptors thereby increasing serotonergic transmission in the hypothalamus and, indirectly, melatonin levels, which would explain its 73% efficacy in the treatment of HH⁸. However, due to lithium's many side effects, the preferred first line option is caffeine with efficacy of 53%⁸. In the case of our patient special attention was given to the fact that the Solpadein, which was most effective at managing her headaches, contained 30 mg of caffeine per tablet. She was successfully treated with a cup of coffee every night before bed, and this led to the *ex juvantibus*

confirmation of the diagnosis of HH. Caffeine acts by interacting with adenosine receptors and thereby causing wakefulness and, additionally, anti-nociception. Contrary to widely held belief among patients, it rarely disrupts their sleep¹². Substantial treatment response to caffeine seems to be pathognomonic for HH diagnosis with many patients discovering its effectiveness on their own before being diagnosed, similarly to our patient¹². Other treatment options include melatonin and indomethacin with comparable efficacy.

Conclusion

HH is an obscure headache disorder albeit a debilitating one. It is a diagnosis of exclusion due to its notable overlap with other, more common, primary headache disorders. However, HH should be an important diagnosis to consider due to the dissimilarity in its treatment strategy in comparison to other primary headache disorders.

Bibliography

1. Raskin NH. The Hypnic Headache Syndrome. *Headache: The Journal of Head and Face Pain*. 1988 Sep;28(8):534–6.3.
2. Gobel H. 4.9 Hypnic headache [Internet]. ICHD-3. Available from: <https://ichd-3.org/other-primary-headache-disorders/4-9-hypnic-headache/>
3. Melchior AG, Ayyoub A, Christensen RH, Al-Khazali HM, Amin FM, Ashina H. Epidemiology and clinical features of hypnic headache: A systematic review and meta-analysis. *Cephalalgia: An International Journal of Headache* [Internet]. 2023 Dec 1 [cited 2024 May 12];43(12):3331024231218389. Available from: <https://pubmed.ncbi.nlm.nih.gov/38051816/#:~:text=The%20pooled%20mean%20age%20of>
4. Kryger MH, Roth T, Goldstein CA, Dement WC. *Principles and practice of sleep medicine*. Philadelphia, PA: Elsevier; 2022.
5. Orr SL, Friedman BW, Christie S, Minen MT, Bamford C, Kelley NE, et al. Management of Adults With Acute Migraine in the Emergency Department: The American Headache Society Evidence Assessment of Parenteral Pharmacotherapies. *Headache: The Journal of Head and Face Pain*. 2016 Jun;56(6):911–40.
6. De Simone R, Marano E, Ranieri A, Bonavita V. Hypnic headache: an update. *Neurological Sciences*:

Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology [Internet]. 2006 May 1 [cited 2021 Sep 23];27 Suppl 2:S144-148. Available from:

[https://pubmed.ncbi.nlm.nih.gov/16688619/#:~:text=Hypnic%20headache%20\(HH\)%20is%20a](https://pubmed.ncbi.nlm.nih.gov/16688619/#:~:text=Hypnic%20headache%20(HH)%20is%20a)

7. Lindner D, Scheffler A, Nsaka M, Holle-Lee D. Hypnic Headache - What do we know in 2022? *Cephalalgia: An International Journal of Headache* [Internet]. 2023 Mar 1;43(3):3331024221148659. Available from: <https://pubmed.ncbi.nlm.nih.gov/36786376/>

8. Silva-Néto RP, Santos PEMS, Peres MFP. Hypnic headache: A review of 348 cases published from 1988 to 2018. *Journal of the Neurological Sciences*. 2019 Jun;401:103–9.

9. Evers S, Goadsby PJ. Hypnic headache: Clinical features, pathophysiology, and treatment. *Neurology*. 2003 Mar 25;60(6):905–9.

10. Holle D, Naegel S, Krebs S, Gaul C, Gizewski ER, Diener H, et al. Hypothalamic gray matter volume loss in hypnic headache. *Annals of Neurology*. 2010 Nov 8;69(3):533–9.

11. Boes AD, Fischer D, Geerling JC, Bruss J, Saper CB, Fox MD. Connectivity of sleep- and wake-promoting regions of the human hypothalamus observed during resting wakefulness. *Sleep*. 2018 May 29;41(9).

12. Holle D, Obermann M. Hypnic headache and caffeine. *Expert review of neurotherapeutics*. 2012 Sep 1;12(9):1125–32.

VI. Public Health and Health Management Session

The role and impact of telemedicine in reinventing healthcare management

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Introduction: Telemedicine has the potential to improve healthcare access and streamline physicians' workload. Despite its increased use, there is a lack of regulation, opening up questions about patient safety and guidelines for physicians. **Aim:** The aim of this review is to examine the benefits and drawbacks of telemedicine in its application in primary healthcare. **Materials and methods:** A search was conducted in Google Scholar, PubMed, and ScienceDirect Database as well as official webpages of governmental organisations by using keywords and word combinations. **Results and discussion:** In the digital age, there is an increasing reliance on technology to improve and enhance communication and the healthcare sector is no stranger to this. Studies have shown that since the advent of the Covid-19 pandemic, there has been an uptick in use of telemedicine consultations for diagnosis, management, and patient reviews in primary healthcare. Modern society in the West favours quick and easy access to all aspects of life, including medicine, and in several surveys reviewed, patients have shown a favourable opinion of telemedicine. There are, however, important limitations to telemedicine that mustn't be ignored due to the risk of patient harm. **Conclusion:** Telemedicine is a new approach to primary healthcare, providing patients in remote areas access to quality medical opinions that they would otherwise have been without. However, in this fast-paced digital world, more research is needed to clarify telemedicine's limitations so appropriate guidelines for its use can be drawn up to protect patient safety.

Keywords: *Telemedicine, primary healthcare, chronic diseases, diagnosis*

Introduction

Telemedicine is “the use of telecommunication and information technology for the purpose of providing remote health assessments and therapeutic interventions” (1). It can be categorised into two types: synchronous and asynchronous telemedicine. Synchronous telemedicine uses audiovisual technology to allow for real-time communication between patient and practitioner. The first instances of this were performed via closed-circuit television but today, high-speed internet facilitates this type of telemedicine. Whereas asynchronous telemedicine follows a store-and-forward model; patient data such as radiological images and dermatological photographs, are taken and then sent to a professional for assessment.

The millennium marked the arrival of the technological age. Over the last twenty-five

years there have been many technological developments, and the proliferation of these technologies has also resulted in a decrease in their cost. The affordability of technology now means that ownership in Europe of smartphones and laptops connected to the Internet is widespread; GSMA estimates that by 2030 92% of Europe will own a smartphone and 87% of these connections will be 5G (2). With the Internet at most people's fingertips at practically any hour of any day, access to information and inevitably medical information is quick and easy. Since the Internet was invented in 1983, the first medical help webpages emerged in the late 1990s (3), now there are over 70,000 medical websites (4). The people of today are used to instant access to not only information but also services – ordering food, delivery of groceries, booking holidays – therefore the

persistent analogue nature of many healthcare systems in the world can seem outdated, slow and inefficient. Telemedicine has the potential to bring elements of healthcare in line with these twenty-first century expectations.

Although its use can be dated back to the mid-twentieth century, telemedicine was introduced more broadly into healthcare in 2012. However, it was the COVID-19 pandemic that proved to be the catalyst for accelerated advancement and integration of telemedicine.

Aim

Through this review, the vast advantages and disadvantages of telemedicine will be examined to better understand its limitations and therefore how its application can be improved. The main focus of this review is specifically on synchronous telemedicine, as it was found that this was the type that saw the greatest increase in use during the pandemic.

Methods

A scientific report was conducted to identify relevant English language articles on telemedicine with a focus on its utilisation and impact; including during the COVID-19 pandemic and after. PubMed, Google scholar, ScienceDirect, and other scientific databases were searched using keywords such as “telemedicine”, “telehealth”, “COVID-19”, “healthcare outcomes”, “access”, and “health equity”. The search included articles published up to the current date to ensure the inclusion of recent research. The inclusion criteria consisted of studies published in peer-reviewed journals, meta-analyses, systematic reviews, and articles with a primary focus on telemedicine.

Data was extracted from selected articles: information on the growth of telemedicine before, during, and after the COVID-19 pandemic; regulatory changes; payment policies; patient and provider satisfaction; and

outcomes. Specific emphasis was given to identifying key findings related to the benefits of telemedicine for patients and healthcare providers, comparisons between telemedicine and in-person care, and cost-saving implications.

Results and discussion

A total of 43 articles were identified from our research, of which 5 were included as they focused on the role and impact of telemedicine in healthcare. The results from the data analysis consistently showed us repeating themes of:

- The increased usage of telemedicine especially during and post the COVID-19 pandemic.
- Cost saving implications of telemedicine.
- Comparison between telemedicine and in person care.
- Strategies for educating the healthcare workforce.
- The benefits of telemedicine for patients and healthcare provider.

The increased use of telemedicine during and after COVID-19

The COVID-19 pandemic presented new challenges for healthcare. Healthcare providers had to adapt their usual methods of providing care in a such a way that both parties were safe and patients still felt that their healthcare needs were being met. Telemedicine proved to be an invaluable part of these new practices.

An independent study was conducted and data from a sample size of 36 million working US citizens was obtained. It showed that over a span of 3 months in 2019, from March to June, there was a 766% increase in telemedicine usage (5). Healthcare providers were switching from traditional in-person appointments to online appointments, to ensure patients were being seen in a safe way. Out of 2000 patients, only 14% of them

stated that they had had a telemedicine appointment before the COVID-19 pandemic (6). However, it was found that during and after the pandemic, the number of patients using telemedicine was 57% and of those with chronic illnesses, the incidence was even higher, at 77% (6). It is clear that telemedicine gave healthcare workers the ability to remotely see patients suspected of having COVID, and other illnesses; thus protecting themselves and at-risk groups in the clinical setting. The video consultations offered by synchronous telemedicine allows the healthcare professionals to take an anamnesis, order any tests, define treatments, and offer regular monitoring which in turn, improves patient cooperation. The ability to perform all of these tasks remotely means that the patient is called in only when deemed absolutely necessary. The fact that a huge proportion of patients would still prefer to continue to use telemedicine post pandemic, suggests that there must be other factors leading to patients opting for telemedicine. Testimonies suggest that patients find telemedicine to be efficient and effective, supporting the idea that, depending on the circumstances, there may no longer be a need for healthcare professionals to see all patients in person. Further insight into some of the circumstances in which the healthcare system can utilise synchronous telemedicine to streamline patient interactions can be seen in the testimonies below:

Testimony 1: “Yes, I prefer to be examined remotely, but it's a matter of gratitude. Even if you have a minor illness, when the doctor treats you, it seems like she saved your life. I would like to see the doctor with whom I have good communication once a month.” (7)

Testimony 2: “It should be better to first meet online, give the necessary examinations to the hospital, and then meet online to talk about the results.” (7)

Testimony 1 and 2 suggest that some patients prefer to be seen or examined remotely at first, and then go into the hospital or at the clinic to be seen only if required. Although testimony 1 claims that the patient would opt to be seen remotely, it does allude to the comfort factor of seeing a doctor in person enables them to express themselves more freely and openly. Unfortunately, this is an unavoidable limitation of telemedicine as the screens, on both sides, form a barrier between the patient and the physician.

Testimony 3: “To go to the hospital for testing and waiting for the result had taken all day before, it was time consuming even though I lived close to the hospital. Now, if my doctor orders some tests after video talk, I go to the hospital and register, give the tests, and go wherever I want. After some hours I asked my doctor if the results were available. Then we chat about them, and I get the prescription my doctor signed electronically via my personal health system called e-Nabiz [name for governmental website for personal health data (meaning e-Pulse)]” (7).

This testimony highlights arguably the greatest attribute of telemedicine – its ability to reduce the time patients spend interacting with the healthcare system. Decreasing the number of trips to the hospital means that the patient saves both time and money that would otherwise be spent on transport and lost on work absences. Instead, they would be notified about when to attend hospital for tests and once the results of the tests were ready, they would be able see an explanation of their results online.

The 3 testimonies above clarify why telemedicine has remained popular with patients even after pandemic regulations were lifted. Physicians can use telemedicine to see patients with a range of ailments that do not need to be seen in person. Should the

physician require further patient testing, the patients can be advised to come to the hospital or clinic and be seen. The burden faced by the healthcare workers has been increasing exponentially making time extremely valuable. By decreasing the number of patients seen in person, this leaves more time for physicians to attend to the needs of patients with more serious and life-threatening conditions.

Telemedicine's cost saving impact

Telemedicine has eased the cost burden on healthcare and has been incorporated for both acute and chronic disease management. Conditions such as pharyngitis, uncomplicated cystitis, COPD and hypertension have been followed up using telemedicine and studied (5). During the year of 2020, 20% of all patients in the US, were seen using telemedicine, which accounted for \$29 billion of the medical services (6) of the total \$4.124 trillion cost (8). This implies that only 0.7% of the total budget was spent on telemedicine, for 20% of the patient population. To put this into perspective, using traditional methods the cost should have amounted to \$824.8 billion, making this a huge budgetary success. The money saved by using telemedicine can then be reallocated and used in other sectors of healthcare that would benefit from additional funding.

Telemedicine allows better follow up and routine checking for patients with both acute and chronic conditions. By using telemedicine effectively, it can lead to a decrease in the number of emergency department admissions (5). Studies have also shown that follow up appointment durations roughly accounted for 2–3 minutes of the physician's time (5), whereas patients that went to the hospital or clinics would take much longer. Not only this, patients would have to apply to take time off work and change their schedules in order to accommodate for healthcare appointments.

Testimony 4: "It would be best if it were widespread in Turkey. It is so good for our citizens. No travel cost, no other expenses. You sit home and talk face-to-face to the doctor, even with professors, via your webcam. What more do we want? Even the remote physiological support of the doctor helps with the cure." (7)

This patient testimony suggests that, from an individual perspective, in order to maintain their health, there are hidden costs involved. This can hugely disadvantage those that are of lower income and lead them to seek medical attention at a much later stage, when disease regression may not be possible, further burdening the healthcare system.

Rural living and disabled patients

For those patients living in rural environments with difficulties in accessing healthcare, telemedicine is a portal for them to obtain the medical attention that they need in a timely manner. The only requirement necessary is a stable internet connection. Rural communities may lack staff and resources making it more difficult for patients and for the healthcare providers. Not only this, but patients may also need to travel far just for a medical opinion.

Testimony 5: "It ought to be continued after the pandemic. Because I need to travel 3 h for the farthest hospital, but I know people who travel 5–6 h for the same purpose. Remote access is very good for them, too." (7)

The testimony above highlights that patients may need to travel for hours, and that can lead to their condition becoming more critical as the time elapses. Patients in rural areas generally only seek out medical care in life threatening and emergency conditions as time and money spent on travel may seem exorbitant when used for annual check-ups and minor symptoms. Ultimately this disadvantages the rural patient, as late-stage

presentations can lead to a poorer prognosis and increase the likelihood of complications. More specialist medical attention is then required, placing a greater burden on medical staff and costing the healthcare system more in comparison to if it had seen the patient earlier.

One may presume that in poorer or more rural communities, internet connectivity and user ability to connect to the internet may be an obstacle, however studies have shown that this is not the case. Limitations, from a technological perspective “were reported at low rates, much lower than common perceptions” (9). This suggests that technology can be successfully used in more rural communities and allow citizens with proper access to healthcare. Nevertheless, there are still some questions that remain unanswered. For example, do all rural communities have access in some way, shape or form to the internet? Do they have enough experience in using the internet? Would they require some training to use telemedicine?

Regarding those with disabilities, telemedicine allows a way for patients to be seen promptly by their physician without requiring them to leave their home. Disabilities are not limited to only physical impairments; patients may have neurocognitive deficits. Disabled patients may continue to lead an independent life; however, many are dependent on their care provider. It may be difficult for them to attend traditional in-person appointments, due to their individual afflictions. Telemedicine allows the patients to be seen in their familiar and comforting environment and for the physicians to assess them whilst in a calm state.

Although it is evident that telemedicine can be used by those in rural environments and those with disabilities, the question still stands as to how accurate the medical evaluation is.

Strategies for educating the healthcare workforce

Incorporating telemedicine more into everyday healthcare practices will improve the efficiency and effectiveness of the healthcare provided. An American study has shown that 28% of general medicine patients and 53% of chronic illness patients found that the care was the same or better in comparison to in person medical treatment (6). This suggests that patients are opting for and prefer, in some instances, to use telemedicine. The implementation of telemedicine has been gradual, except between the years 2019 and 2020, when a 38% increase was seen (6). This rate however, does not match the demand of patients wanting to be seen or spoken to by a physician. Clinicians have stated that their lack of training has impacted their ability to use telemedicine, with many not feeling confident (5).

As the prevalence of telemedicine is rising to meet the demands of the healthcare system, guidance on its instruction and use should be incorporated into the compulsory training post-graduation for all doctors. Doctors should be required to take regular information technology courses to ensure that their skills are up to date, and that their competency is adequate. Moreover, the patient should be asked whether they are comfortable to use telemedicine methods for their health. Surveys and questionnaires can be used to gauge their understanding. To educate them, short and user-friendly instructional videos can be made available to guide them on what they need to do from their side, what is expected, and what the outcomes may be. This way, both sides are best equipped to use telemedicine.

Testimony 6: “That technique must be widespread one million percent. We live in the technology era. I believe it ought to be more than this. Time is so precious. Especially for doctors, they deal with lives.” (7)

By embracing the technological advancements of the modern era, the healthcare system can help to see many more patients, in a shorter period of time. With most patients expressing a desire for telemedicine to become a normalised part of healthcare management, this should encourage doctors to introduce it into their practice.

Conclusion

Telemedicine is an expanding field in primary healthcare that offers a plethora of benefits to both healthcare providers and to patients. Its increased usage was seen especially during and post the COVID-19 pandemic, highlighting its effectiveness and how it could be efficiently utilised in the future. The cost of healthcare is a burden seen throughout the world, and there are indications that telemedicine could help to combat this. Telemedicine also allows those living in more geographically challenging areas with access to medical expertise, particularly for those living in rural areas. People with disabilities with decreased abilities for travel have also seen many benefits of using telemedicine. Although, it is evident that there are many benefits to the use of telemedicine, the limitations of telemedicine need to be further explored and adequate guidelines need to be set in place to protect the patient's safety.

The modern era of technological advances means that most of the world has the Internet at the fingertips. In spite of this, it should not be taken for granted. Healthcare professionals should, regardless, continue to educate the population with health promotion and disease prevention, as prevention is always better than cure. Outreach programmes and community healthcare education should remain the forefront of their mission. Telemedicine can be further incorporated to help

with this task. Overall, it is evident that there are many benefits to the use of telemedicine, and it seems its full potential has not yet been realised. Even so, whilst in its infancy, the limitations of telemedicine need to be further explored so that adequate guidelines can be drawn up and set in place to protect the safety of patients and providers.

Bibliography

1. NHS Data Model and Dictionary. Telemedicine [Internet]. www.datadictionary.nhs.uk. 2024 [cited 2024 Apr 30]. Available from: www.datadictionary.nhs.uk/nhs_business_definitions/telemedicine.html
2. GSMA. The Mobile Economy Europe 2023 [Internet]. The Mobile Economy. [cited 2024 Apr 30]. Available from: www.gsma.com/solutions-and-impact/connectivity-for-good/mobile-economy/europe
3. Shah A. Simple diagnosis, treatment and prescriptions now a click away [Internet]. NJ Top Docs | Find the Best NJ Doctors and NJ Dentists in New Jersey. 2012 [cited 2024 Apr 30]. Available from: <https://njtopdocs.com/simple-diagnosis-treatment-and-prescriptions-now-a-click-away/>
4. Sbaffi L, Rowley J. Trust and Credibility in Web-Based Health Information: A Review and Agenda for Future Research. *Journal of Medical Internet Research* [Internet]. 2017 Jun 19 [cited 2024 Apr 30];19(6):e218. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC5495972/
5. Shaver J. The State of Telehealth Before and After the COVID-19 Pandemic. *Primary Care: Clinics in Office Practice* [Internet]. 2022 Apr 25 [cited 2024 Apr 30];49(4):517–30. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC9035352/
6. Doximity. 2020 State of Telemedicine Report Examining Patient Perspectives and Physician Adoption of Telemedicine Since the COVID-19 Pandemic [Internet]. 2020 Sep [cited 2024 Apr 30]. Available from: <https://c8y.doxcdn.com/image/upload/Press%20Blog/Research%20Reports/2020-state-telemedicine-report.pdf>
7. Altinisik Ergur G, Nuhoglu S, Cobanoglu C, Sengul M, Eryildiz N, Ergur A. The Patient Perspective of Telemedicine in the Context of COVID-19 Pandemic. *Bulletin of Science, Technology & Society*. 2022 Apr 12;42(1-2):39–53.
8. Rama A. Policy Research Perspectives [Internet]. 2022 May [cited 2024 Apr 30]. Available from: <https://www.ama-assn.org/system/files/prp-annual-spending-2020.pdf>
9. Butzner M, Cuffee Y. Telehealth Interventions and Outcomes Across Rural Communities in the United States: Narrative Review. *Journal of Medical Internet Research* [Internet]. 2021 Aug 26 [cited 2024 Apr 30];23(8). Available from: www.jmir.org/2021/8/e29575/

Approaches to increase physical activity – good practices for the prevention and assessment of musculoskeletal dysfunctions

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Introduction: Using prevention to increase health benefits and achieve a higher level of population well-being is one of the goals of health care. Physical activity (sports, fitness training or various movement practices) is one of the main factors for primary health prevention. It is a determining factor in the fight against hypodynamia. According to Eurobarometer data from 2022, nearly half (48.3%) of young people in the low union lead a sedentary lifestyle. The set of the study is the detection of musculoskeletal disorders and the application of a program based on the Pilates method to improve motor activity in case of impaired posture. **Materials and methods:** The study covered 120 persons aged 5–57 years, it was conducted in November 2023 in the city of Plovdiv. It is used with sociological methods, history, examination in combination with high-quality tests for musculoskeletal disorders. **Discussion and results:** The main reasons for musculoskeletal dysfunctions are immobility and incorrect posture. Pathokinesiological analysis showed dysfunctions mainly in the back, neck and shoulders. A program based on the Pilates method was drawn up in accordance with the findings of the functional examination. As a result of the systemic physical loads, positive structural-functional changes of the impaired function have been achieved. Kinesitherapeutic means are applied for the prevention of spinal distortions. **Conclusion:** Time spent in health-enhancing physical activity to improve physical and mental health is an investment in health care, which is why it is necessary to move to a model of healthy behavior and good practice.

Keywords: physical activity, musculoskeletal dysfunctions

Introduction

Measures to increase the physical activity of the population are one of the most economical and effective approaches for better public health in response to the main risk factors, such as unhealthy diet and inactivity [1].

In today's times, physical activity is significantly challenged due to various factors such as technological advances, prolonged time spent in front of the computer, and lack of education about the importance of regular physical activity. Approaches to promoting physical activity have changed dramatically in recent years. For several decades, almost all strategies have been designed to educate and motivate young people to be physically active in order to promote health and prevent a number of diseases. The prevention of postural and spinal disorders is an important factor in public health care due to their significant increase [2].

The Pilates method is a modern mix of physical exercises, also called new fitness gymnastics [3]. Motor practice helps shape the figure, control weight, builds muscle strength, endurance, and motor qualities. Physical fitness is the first requisite of happiness. Our interpretation of physical fitness is the maintenance of a uniformly developed body with a sound mind fully capable of naturally, easily, and satisfactorily performing our many and varied daily tasks with spontaneous zest and pleasure (Pilates 1945). In Joseph Pilates' book, *Return to Life Through Contrology*, he writes that his method is not merely a physical fitness regimen of mindlessly repeated exercises, but rather a holistic approach to well-being and a lifelong process of refinement [4].

The study aims to investigate the relationship between reduced physical activity and musculoskeletal dysfunctions.

Materials and methods

The study covered 120 persons aged 5–57 years, it was conducted in November 2023 in the city of Plovdiv. It is used with sociological methods, history, examination in combination with high-quality tests for musculoskeletal disorders.

Results and discussion

Kinesiotherapeutic methods and exercises based on the Pilates method – a modern mix of fitness gymnastics – have been prepared. The purpose of the program is to strengthen the muscles that stabilize the posture and strengthen the new motor patterns.

The Pilates method, developed by Joseph Pilates, is based on integrated exercise to achieve overall health. The principles underlying this method include whole body exercise, balance between stability and mobility, exercise functionality and attention to breathing [5]. The Pilates method is distinguished by a wide assortment of exercises that cover all parts of the body. These exercises progress from basic to more complex levels, allowing practitioners to develop their skills and abilities gradually [6]. The Pilates method focuses on improving the stability and mobility of the body. Pilates exercises help develop the stability of core muscles and stimulate joint mobility, which contributes to improving the body's functionality. Pilates exercises focus on closed and open kinetic chains, allowing practitioners to move in a natural and functional manner. These exercises load the muscles both statically and dynamically, emphasizing concentric and eccentric muscle contractions. Pilates places great importance on proper breathing during exercise. Proper breathing not only helps to stabilize the body, but also helps the circulation of blood and oxygen in the body. The Pilates method is adaptable for different groups of patients and students. It

can be used both for rehabilitation after injury and surgery, and to improve function in people with chronic diseases. The Pilates method is an integrated approach to exercise that focuses on the overall health of the body. Combining a balance between stability and mobility, exercise functionality and proper breathing, Pilates can be an effective tool to improve people's health and quality of life [7]. The human body is an extremely complex system in which each structure plays its essential role in achieving optimal health. In order to maintain this balance, it is essential to identify any imbalances and work to correct them by developing the ability to distinguish movements. Understanding and feeling what parts of the body move, when, how, and in cooperation with others is essential. Each exercise in the Pilates method aims to strengthen certain muscles or muscle groups while others are stretched or relaxed. This complex relationship between different muscle groups helps to develop motor culture, which ultimately helps to prevent future dysfunctions at the locomotor level. When muscles contract, visually they usually shrink. At the same time, when stretched, they lengthen beyond their natural resting length [8]. Due to the fact that muscles can only pull and not repel, stretching requires the respective muscle group to be shortened or a specific body position applied using gravity, body weight or other aids to achieve optimal stretching. In order to achieve the greatest distance between the ends of the muscle, a detailed knowledge of the attachment points of the muscle and correct execution of the technique is necessary to achieve the desired effect [9].

The developed Pilates methodology was presented in an attractive and motivating way for the teenagers, so that they would be interested in continuing the activities even after completing the course.

Evidence is presented in studies that motor activity, physical environment conditions and other factors in children are important for their health in an active age, and Pilates programs can be recommended as an appropriate exercise for impaired posture and musculoskeletal disorders [10].

Conclusion

The prevention of muscle imbalances and distortions of the body is achieved through the systematic application of the Pilates method, which not only strengthens and stabilizes the muscles, but also stretches them and improves their flexibility. Pilates' approach to training the body as a whole, with an emphasis on correct biomechanics and breathing techniques, is the key to preventing muscle imbalances and maintaining an optimal functional state of the body.

References

1. WHO. Global strategy on diet, physical activity and health. Geneva: World Health Organization, 2004. http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf.
2. Koleva G, Davidova S, Durmushalieva S, Kasnakova P. Application of the Pilates Method for Prevention and Control of Postural Disorders and Spinal Curvatures in Adolescents. Science and Youth Conference. Social Medicine. 2023:13-17.
3. Pilates, J. H. Return to life through contrology. Miami, FL: Pilates Method Alliance. 1945.
4. Osar E, M. Bussard. Functional Anatomy of the Pilates Core: An Illustrated Guide to a Safe and Effective Core Training, North Atlantic Books, Berkeley, California, 2015:7-13.
5. Wood S. Pilates for rehabilitation. Champaign, IL: Human Kinetics, 2019:13.
6. Latey P. The Pilates method: history and philosophy. J Body Mov Ther 2001; 5: 275–282.
7. Jurak I, Delaš K, Erjavec L, Stare J, Locatelli I. Effects of Multidisciplinary Biopsychosocial Rehabilitation on Short-Term Pain and Disability in Chronic Low Back Pain: A Systematic Review with Network Meta-Analysis. J Clin Med. 2023 Dec 4;12(23):7489. doi: 10.3390/jcm12237489. PMID: 38068539; PMCID: PMC10707228.
8. Lytras D, Iakovidis P, Sykaras E, Kottaras A, Kasimis K, Myrogiannis I, Barouxakis A, Tarfali G. Effects of a tailored mat-Pilates exercise program for older adults on pain, functioning, and balance in women with chronic non-specific low back pain: a randomized controlled trial. Aging Clin Exp Res. 2023 Dec;35(12):3059-3071. doi: 10.1007/s40520-023-02604-7. Epub 2023 Nov 7. PMID: 37934400.
9. Yamato TP, Maher CG, Saragiotto BT, Hancock MJ, Ostelo RW, Cabral CM, Menezes Costa LC, Costa LO. Pilates for low back pain. Cochrane Database Syst Rev. 2015 Jul 2;2015(7):CD010265. doi: 10.1002/14651858.CD010265.pub2. PMID: 26133923; PMCID: PMC8078578.
10. Lee S, C. Lee, D. O'Sullivan, et al. Clinical effectiveness of a Pilates treatment for forward head posture. Journal of Physical Therapy Science 28 (7) 2016: 2009-13.

Risk factors associated with retinal vascular occlusive diseases

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Introduction: Retinal vascular occlusive diseases are associated with multiple risk factors, including cardiovascular diseases, and can cause visual dysfunction. Generally, they are classified into two types: retinal arterial occlusions and retinal venous occlusions. **Aim:** The study aims to identify risk factors associated with retinal vascular occlusive diseases and evaluate their impact. **Materials and methods:** Forty-eight patients with established retinal vascular occlusive diseases registered between January and December 2023 participated in the study. Registration of the risk factors was done using a questionnaire. **Results:** Among all patients, 81.3% were found to have systemic hypertension, 10.4% – had diabetes, 27.1% – had cardiovascular diseases, 8.3% had glaucoma, and 10.4% had registered malignant disease. Twenty-five percent were found to have dyslipidemia during laboratory tests. It is estimated that 27.1% of them smoke. **Conclusion:** Among the patients who have retinal vascular occlusive diseases, the leading risk factor was hypertension, followed by cardiovascular diseases, dyslipidemia, and smoking. Additionally, age appears to be a significant risk factor.

Keywords: retinal vascular occlusive diseases, risk factors, retinal artery occlusion, retinal vein occlusion

Introduction

In ophthalmology, retinal vascular occlusions are an important group of diseases that cause unilateral vision loss (1). The diseases can be classified into two types: retinal artery occlusion (RAO) and retinal vein occlusion (RVO). In comparison to retinal vein occlusions, retinal artery occlusions occur less frequently (1, 2). It is possible to divide both diseases into central and branch occlusions, depending on the location of the obstruction (2, 3). RAO is considered the ophthalmic variant of acute cerebral ischemia and causes unilateral, sudden, and profound painless vision loss (4). As a result of atherosclerosis and thromboembolism, RVO causes reduced vision mostly due to macular edema and retinal ischemia, with complications such as vitreous hemorrhage, epiretinal membrane formation, neovascularization of the iris, and neovascular glaucoma developing in the later stages (3, 5). Two main groups of risk factors can be differentiated: systemic risk factors and ocular risk factors. Among the systemic risk factors are arterial hypertension, diabetes mellitus, dyslipidemia, cardiovascular diseases, and malignancies, whereas ocular risk factors include glaucoma or ocular hypertension, orbital neoplasms, and thyroid-associated ophthalmopathy (TAO) (6). Atherosclerosis and thromboembolism are commonly associated with retinal

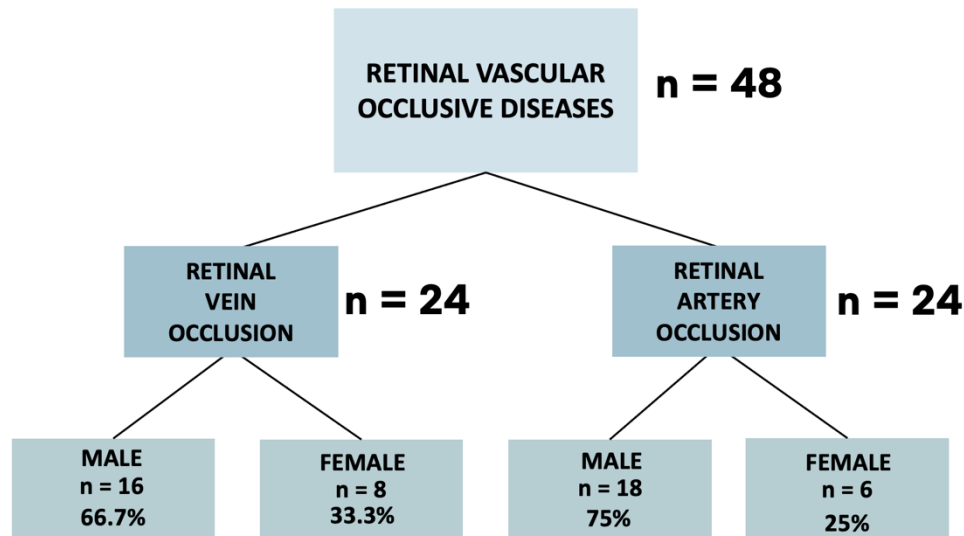
vascular occlusions (2). The risk of emboli associated with RAO is higher in patients with hypercholesterolemia, arterial hypertension, obesity, diabetes mellitus, and atrial fibrillation (2, 7). Patients with RVO are most likely to have arterial hypertension, diabetes mellitus, and hyperlipidemia (2, 6, 8, 9). Also, smoking is an important risk factor (8, 9).

Aim

This study aims to identify risk factors in a cohort of patients with established retinal vascular occlusive diseases and evaluate their impact.

Materials and methods

Forty-eight patients with established retinal vascular occlusive diseases registered between January and December 2023 participated in the study. The 48 patients were categorized into two subgroups: those with established RVO consisting of 24 patients and those with RAO 24. In terms of gender distribution, 66.7% (16 patients) of the patients with established RVO were males and 33.3% (8 patients) were females. On the other hand, 75% (18 patients) of the RAO patients were male, while 25% (6 patients) were female. The mean age for RVO patients was 62.38 ± 14.11 , and 65.71 ± 11.02 for RAO patients. Registration of the risk factors was done using a questionnaire.



Results

This study evaluated 48 patients with established retinal vascular occlusive diseases, and their risk factors were recorded and analyzed. Two subgroups were formed based on the main group as mentioned above. A statistical analysis of these groups was also performed for risk factor percentage ratios.

According to statistical analysis of the registered data, the most common risk factor for retinal vascular occlusive diseases is systemic hypertension (81.3%), followed by cardiovascular diseases (27.1%) and dyslipidemia (25%). Smoking (27.1%) appears to be an important risk factor. **Table 1** and **Figure 1**.

Table 2 and **Figure 2** provide descriptive data about risk factors associated with the

first subgroup of patients with established RVO. Among patients with RVO, systemic hypertension (79.2%) is the most common risk factor. Moreover, established glaucoma or ocular hypertension (16.7) is more common in patients with RVO than in those with RAO. Additionally, cardiovascular diseases (29.2%) and smoking (20.8%) are significant risk factors for RVO.

A descriptive analysis of the second subgroup of patients with established RAO is presented in **Table 3** and **Figure 3**. A noteworthy finding is that hypertension is the most common risk factor, followed by dyslipidemia and smoking. In patients with RAO, there is a low incidence of glaucoma or ocular hypertension.

Table 1.

Risk factors	Retinal vascular occlusions
Systemic hypertension	81.3%
Diabetes mellitus	10.4%
Cardiovascular diseases	27.1%
Glaucoma/ocular hypertension	8.3%
Dyslipidemia	25%
Malignancies	10.4%
Smoking	27.1%

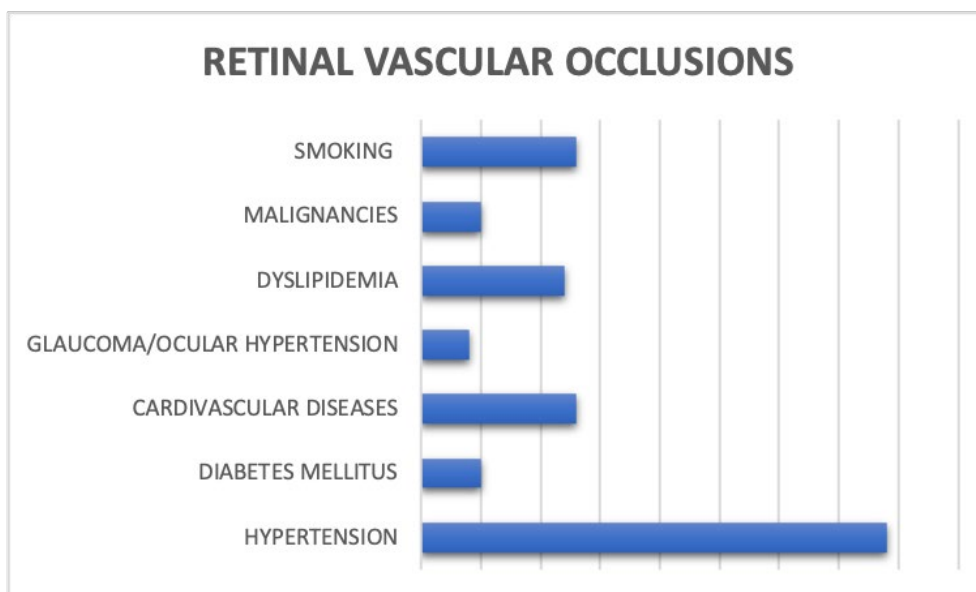


Figure 1.

Table 2.

Risk factors	Retinal vein occlusion
Systemic hypertension	79.2%
Diabetes mellitus	12.5%
Cardiovascular diseases	29.2%
Glaucoma/ocular hypertension	16.7%
Dyslipidemia	16.7%
Malignancies	12.5%
Smoking	20.8%

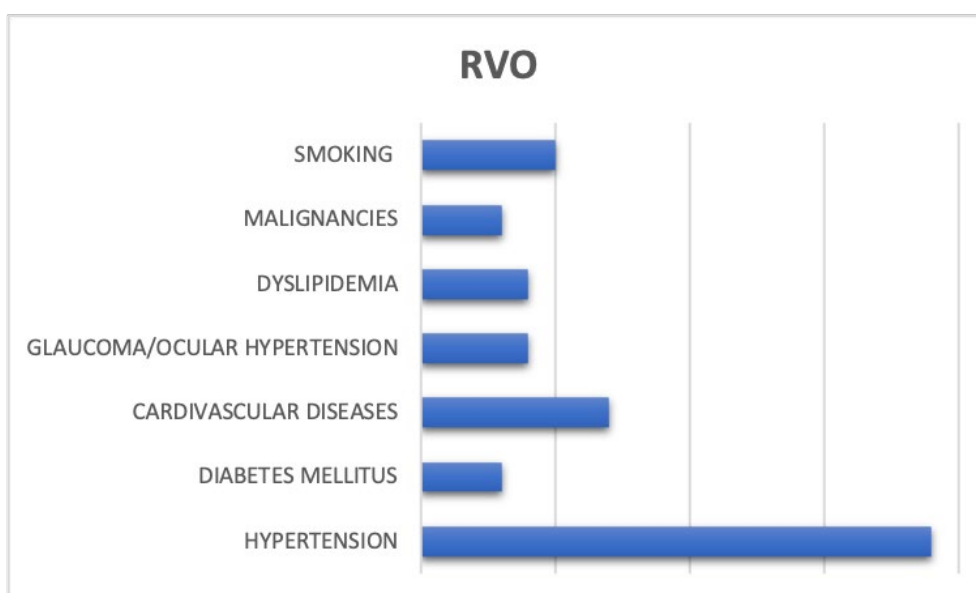
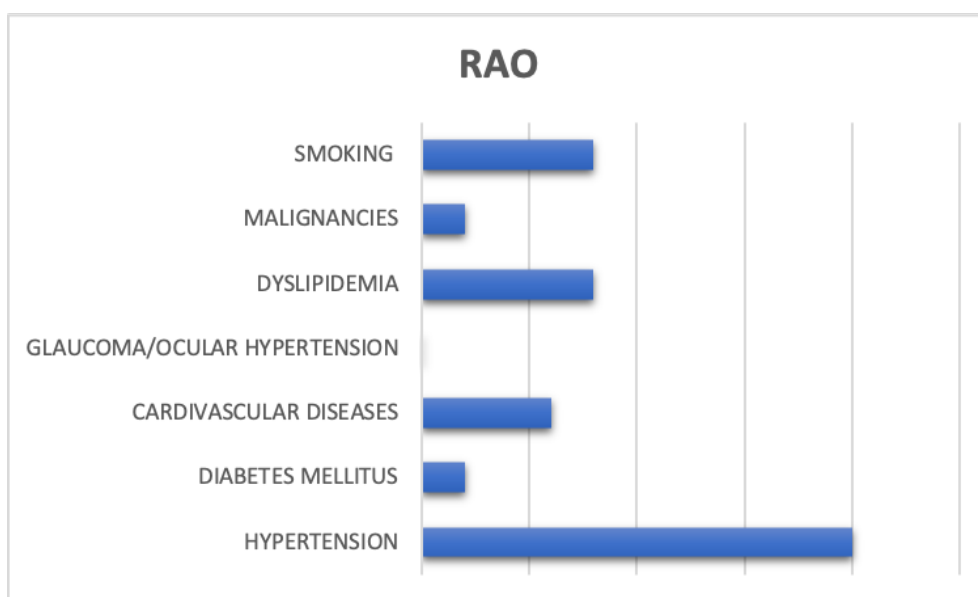


Figure 2.

Table 3.

Risk factors	Retinal arterial occlusion
Systemic hypertension	83.3%
Diabetes mellitus	8.3%
Cardiovascular diseases	25%
Glaucoma/ocular hypertension	0%
Dyslipidemia	33.3%
Malignancies	8.3%
Smoking	33.3%

**Figure 3.**

Discussion

As with other vascular-associated systemic diseases, retinal vascular occlusive diseases are often associated with risk factors like hypertension, dyslipidemia, and cardiovascular diseases such as atrial fibrillation, coronary artery disease, etc. (6, 7, 10). It was found in the present study that systemic hypertension is the leading risk factor for both RVO and RAO. According to the Gutenberg Study (8), hypertension patients are almost twice as likely to develop RVO as those without hypertension. The combination of several risk factors, such as established hypertension combined with dyslipidemia and smoking as a contributing risk factor, is critical (10–12).

It has been reported that men are more likely to suffer from retinal vascular occlusive diseases than women (9–11). According to the Gutenberg Health Study, men are affected 1.7 times more often by RVO than women (9). According to other studies, one gender does not predominate over another (12). Among both subgroups in the present study, males outnumbered females. Age correlates with RVO prevalence – as people age, their frequency increases, so age is considered one of the most significant risk factors (13). Atherosclerotic changes in arterial vessels, including retinal arteries, are more prominent in elderly people, which might explain this observation. Consequently, the venous retinal vessels are compressed at the

crossing points, disrupting the venous vascular wall (14). It was estimated that the mean age of the patients with established retinal vascular occlusive disease in the present study was around 60.

It is not fully understood how elevated intraocular pressure can cause RVO in patients with ocular hypertension or diagnosed glaucoma. However, it is believed that pressure-induced mechanical and hemodynamic changes act at the level of the lamina cribrosa and promote vascular changes (15). The presence of glaucoma and ocular hypertension was found to be a greater risk factor for the development of RVO than RAO, and the presence of this comorbidity was not recorded in patients with RAO. Glaucoma has been identified as one of the most important ocular risk factors for RVO (6).

In addition to retinal vascular occlusive diseases, patients with these conditions are at risk of developing future cardiovascular and cerebrovascular incidents such as myocardial infarctions and strokes. Undiagnosed risk factors are the most common cause of these patients' conditions (16–18).

Conclusion

Consequently, systemic hypertension, cardiovascular diseases, dyslipidemia, and smoking are the most common risk factors for retinal vascular occlusive diseases. Furthermore, diagnosis and treatment of underlying risk factors may also prevent occlusive retinal diseases and the development of more severe cardiovascular and cerebrovascular events.

Reference

1. Scott IU, Campochiaro PA, Newman NJ, Biousse V. Retinal vascular occlusions [Internet]. *www.thelancet.com*. 2020. Available from: *www.thelancet.com*
2. Terao R, Fujino R, Ahmed T. Risk Factors and Treatment Strategy for Retinal Vascular Occlusive Diseases. *J Clin Med*. 2022 Oct 27;11(21):6340.

3. Pulido JS, Flaxel CJ, Adelman RA, Hyman L, Folk JC, Olsen TW. Retinal Vein Occlusions. *Ophthalmology*. 2016 Jan 1;123(1):P182–208.
4. Dattilo M, Newman NJ, Biousse V. Acute retinal arterial ischemia. Vol. 3, *Annals of Eye Science*. AME Publishing Company; 2018.
5. Macdonald D. The ABCs of RVO: A review of retinal venous occlusion. Vol. 97, *Clinical and Experimental Optometry*. Blackwell Publishing Ltd; 2014. p. 311–23.
6. Kolar P. Risk factors for central and branch retinal vein occlusion: A meta-analysis of published clinical data. Vol. 2014, *Journal of Ophthalmology*. Hindawi Publishing Corporation; 2014.
7. Rudkin AK, Lee AW, Chen CS. Vascular risk factors for central retinal artery occlusion. *Eye*. 2010;24(4):678–81.
8. Ponto KA, Scharrer I, Binder H, Korb C, Rosner AK, Ehlers TO, et al. Hypertension and multiple cardiovascular risk factors increase the risk for retinal vein occlusions: Results from the Gutenberg Retinal Vein Occlusion Study. In: *Journal of Hypertension*. Lippincott Williams and Wilkins; 2019. p. 1372–83.
9. Ponto KA, Elbaz H, Peto T, Laubert-Reh D, Binder H, Wild PS, et al. Prevalence and risk factors of retinal vein occlusion: The Gutenberg Health Study. *Journal of Thrombosis and Haemostasis*. 2015 Jul 1;13(7):1254–63.
10. Stem MS, Talwar N, Comer GM, Stein JD, Author Joshua Stein CD. A Longitudinal Analysis of Risk Factors Associated With Central Retinal Vein Occlusion. *Ophthalmology* [Internet]. 2013 [cited 2023 Apr 20];120(2):362–70. Available from: *http://aaojournal.org*
11. Callizo J, Feltgen N, Pantenburg S, Wolf A, Neubauer AS, Jurklics B, et al. Cardiovascular Risk Factors in Central Retinal Artery Occlusion: Results of a Prospective and Standardized Medical Examination. *Ophthalmology*. 2015 Sep 1;122(9):1881–8.
12. Klein R, Moss SE, Meuer SM, Klein BEK. The 15-Year Cumulative Incidence of Retinal Vein Occlusion The Beaver Dam Eye Study [Internet]. Vol. 126, *Arch Ophthalmol*. 2008. Available from: *https://jamanetwork.com/*
13. Rogers S, McIntosh RL, Cheung N, Lim L, Wang JJ, Mitchell P, et al. The Prevalence of Retinal Vein Occlusion: Pooled Data from Population Studies from the United States, Europe, Asia, and Australia. *Ophthalmology*. 2010;117(2).
14. ERDOĞAN H. Retinal Ven Dal Oklüzyonu Olan Hastalarda Risk Faktörleri. *Mustafa Kemal Üniversitesi Tıp Dergisi*. 2019 Mar 26;10(36):10–3.
15. Frucht J, Shapiro A, Merin S. Intraocular pressure in retinal vein occlusion [Internet]. Vol. 68, *British Journal of Ophthalmology*. 1984. Available from: *http://bjo.bmj.com/*
16. Lavin P, Patrylo M, Hollar M, Espallat KB, Kirshner H, Schrag M. Stroke Risk and Risk Factors in Patients With Central Retinal Artery Occlusion. *Am J Ophthalmol*. 2018 Dec 1;196:96–100.

17. Ho J der, Liou SW, Lin HC. Retinal Vein Occlusion and the Risk of Stroke Development: A Five-year Follow-up Study. *Am J Ophthalmol.* 2009;147(2).

18. Li M, Hu X, Huang J, Tan Y, Yang B, Tang Z. Impact of retinal vein occlusion on stroke incidence: A meta-analysis. Vol. 5, *Journal of the American Heart Association.* John Wiley and Sons Inc.; 2016.

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VII. Dental Medicine Session

The application of gingival mesenchymal stem cells in periodontal regeneration – literature review

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Introduction: Periodontal regeneration is the most desired outcome of periodontal treatment, but it cannot always be achieved. A variety of approaches and materials are used to achieve this goal. In recent years, the application of mesenchymal stem cells (MSCs) and in particular gingival mesenchymal stem cells (GMSCs) to achieve regeneration of periodontal tissues has been widely discussed. **Objective:** The aim of this review is to analyze the clinical benefit of using gingival mesenchymal stem cells to support the regeneration of affected periodontal tissues, by reviewing specialized scientific literature. **Methods:** Full articles written in English and published in peer-reviewed scientific journals were analyzed. **Results:** Dental tissue-derived mesenchymal stem cells (MSCs), especially those from the gingiva, have attracted attention in the field of regenerative medicine due to their provenance, rapid proliferation rates, and genomic stability. Experiments show that the application of these cells helps the regeneration of periodontal tissue, including the formation of new periodontal ligaments and bone. Although there are clinical trials on the use of these cells in the treatment of periodontitis, the results are still unclear. **Conclusion:** Gingival mesenchymal stem cells are considered a powerful tool for regenerative therapy in periodontitis. Despite the promising results of the studies, in order to move to clinical trials, further efforts are needed to establish standardized procedures for the production of GMSCs, the selection of suitable donors and the identification of the right patient groups for treatment.

Keywords: periodontal regeneration, gingival mesenchymal stem cells, periodontitis

Introduction

Periodontitis is an infectious inflammatory disease and is characterized by periodontal pocket formation, attachment loss and alveolar bone resorption. Periodontal therapy aims to achieve periodontal regeneration by restoring connective tissue, cementum, and alveolar bone (1). Periodontal regeneration based on mesenchymal stem cells (MSCs) represents a promising approach to improve periodontal health. MSCs are found in almost all tissues in the human body, including extraoral and intraoral tissues, and are easily extracted (2). These cells have a high potential for differentiation into various cell types, including those required to regenerate lost periodontal tissue (3), (4). MSCs can be isolated with ease from a variety of dental tissues, including dental pulp stem

cells, apical papilla stem cells, dental follicle stem cells, human exfoliated deciduous tooth stem cells, periodontal ligament stem cells, gingival stem cells, and stem cells derived from tooth germs (5). Gingival mesenchymal stem cells (GMSCs) can be isolated from normal or inflamed gingiva, from attached and free gingiva, and from hyperplastic gingiva (6, 7) and have shown significant potential for periodontal tissue regeneration (8). Genes related to keratinization, the formation of epithelial cells and ectoderm, as well as immune and/or inflammatory reactions are highly expressed in the gingival tissue (9). Scientific research shows that GMSCs can be applied in different ways in periodontal therapy, such as transplantation, injection directly into the affected tissue or in combination with biomaterials, taking

into account the degree of tissue damage, the location of the lesion, as well as individual needs and patient characteristics (10).

Aim

The aim of this review is to analyze the clinical benefit of using gingival mesenchymal stem cells to support the regeneration of affected periodontal tissues, by reviewing specialized scientific literature.

Methods

A search of the electronic databases PubMed, Google Scholar, Medline, ClinicalTrials.gov using keywords periodontal regeneration, gingival mesenchymal stem cells, periodontitis resulted in 113 articles that were screened based on title and abstract relevance to the topic that of interest. Eligibility criteria included in vitro studies and clinical studies in which tissue was obtained only from gingiva. Based on this criterion, 107 were excluded. The full texts of 6 articles were accepted for analysis.

Results

MSCs are multipotent stromal cells with the capacity to differentiate into various cell types, including osteoblasts, adipocytes, and chondrocytes (11). The first stem cells from gingival tissue were derived in 2009 by Zhang et al. (12). In 2016, Górski paid special attention to gingival-derived GMSCs in the oral cavity, stating that they are readily available and can be extracted using minimally invasive surgical techniques (13).

In their study, Tomar GB et al. (14) characterized human gingival tissue-derived stem cells in detail, highlighting that they are easy to isolate, uniformly homogeneous, proliferate faster than bone marrow-derived mesenchymal stem cells BM-MSCs, display a stable phenotype, and maintain normal karyotype and telomerase activity in long-term cultures, are not tumorigenic and can be

generated in a short period of time from gingiva for cell therapy in regenerative medicine.

Studies reporting GMSCs isolation techniques surgically obtained gingival tissue samples from humans using gingivectomy techniques. The gingival tissue was transferred to culture medium supplemented with bovine serum and antibiotics. Tissues were washed with wash buffer and incubated overnight with dispase at 4°C. Dispase was removed from cell suspensions by centrifugation followed by washing with buffer or culture medium. Gingival tissue was digested by collagenase at 37°C for 2 hours. The digested suspension was filtered and centrifuged to separate the cellular fraction. Cells were resuspended in nutrient culture at 37°C in a humidified tissue culture incubator with 5% CO₂ and 95% O₂. When primary cultures become 80%–90% confluent, adherent cells are dissociated and subcultured. A purified population of GMSCs can be obtained 2–3 weeks after initiation of culture (15, 16, 17).

In the periodontitis experimental study conducted by Balaban et al. (18). on rats, reported that topical administration of human gingival mesenchymal stem cells GMSCs for periodontal regeneration contributed to the formation of new periodontal ligament and alveolar bone. In a severe periodontitis model developed in mice Liu X et al. (19) showed that systemic infusion of human GMSCs promoted periodontal tissue regeneration, observing increased alveolar bone height and new bone formation compared to control groups. A similar experiment was conducted by Sun W et al. (20) in which they found that mice transplanted with GMSCs had significantly increased alveolar bone height compared with controls. Also, GMSCs were found in newly formed periodontal ligaments and alveolar bone. The

results of the study show that systemically transplanted GMSCs can integrate into the dental periodontium and promote periodontal tissue regeneration. Another study (21) reported that implantation of autologous GMSCs in the premolar/molar region significantly facilitated periodontal regeneration in a guinea pig periodontal defect model. To this date, there are only two registered clinical trials on the use of GMSCs in the treatment of periodontitis (NCT03137979 and NCT03638154), with no published results yet. In one of the studies, a clinical trial was conducted to evaluate the regenerative potentials of cultured gingival fibroblasts and GMSCs transferred to a beta tricalcium phosphate scaffold in bony periodontal defects in humans by clinical and radiographic parameters. The other aimed to evaluate the safety and efficacy of gingival mesenchymal stem cell therapy for chronic periodontitis in adults by alveolar bone regeneration and other clinical parameters (22).

Discussion

The use of GMSCs represents an intriguing and promising approach in the fight against periodontal diseases. Their easy retrieval is essential. Gingival mesenchymal stem cells can be easily and painlessly isolated from gingival tissue, which is often considered “waste material” after dental procedures. This makes their use convenient and accessible for medical practice.

Furthermore, the ability of GMSCs to differentiate into different cell types provides great potential for their application in periodontal disease therapy. They can transform into cells that are needed to repair lost periodontal tissue, such as osteoblasts to form new bone and fibroblasts to regenerate the periodontal ligament.

Research and experiments in the field of periodontal regeneration also highlight the

potential of gingival mesenchymal stem cells. These studies demonstrate their ability to stimulate regenerative processes in the periodontium by forming new periodontal ligaments and alveolar bone. Such results are essential for future treatment methods of periodontal diseases.

However, further research and clinical trials are needed to determine the exact route for their use in clinical practice and to establish standard procedures for their use in the treatment of periodontal diseases.

Conclusion

Since the initial isolation and characterization of GMSCs, an increasing number of studies have demonstrated their potent regenerative therapeutic potential. Overall, these studies have shown the promising potential of GMSCs-based regenerative therapy for the treatment of periodontitis. However, much more effort is needed to establish the pathway for clinical trials of GMSCs, including selection of appropriate donors, establishment of standard operating procedures for all steps in the production of GMSCs, use of appropriate approaches to prepare GMSCs prior to application, and identification of appropriate patient groups for treatment.

References

1. Hynes K, Menicanin D, Gronthos S, Bartold PM. Clinical utility of stem cells for periodontal regeneration. *Periodontol* 2000, 2012; 59:203–227.
2. Campagnoli C, Roberts I.A.G, Kumar S, Bennett P.R, Bellantuono I, Fisk N.M. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood*, 2001; 98(8):2396-402.
3. Tassi S, Sergio N, Misawa M, Villar C. Efficacy of stem cells on periodontal regeneration: systematic review of pre-clinical studies. *J Periodontal Res*, 2017; 52(5):793-812.
4. Lee YC, Chan YH, Hsieh SC, Lew WZ, Feng SW. Comparing the osteogenic potentials and bone regeneration capacities of bone marrow and dental pulp mesenchymal stem cells in a rabbit calvaria bone defect model. *Int J Mol Sci*, 2019; 20(20):5015

5. Ercal P, Pekozer GG, Kose GT. Dental Stem Cells in Bone Tissue Engineering: Current Overview and Challenges. *Adv Exp Med Biol*, 2018; 1107:113-127.
6. Fawzy El-Sayed KM, Dörfer C, Fändrich F, Gieseler F, Moustafa MH, Ungefroren H. Adult mesenchymal stem cells explored in the dental field. *Adv Biochem Eng Biotechnol*, 2013;130:89–103.
7. Apatzidou D, Nile C, Bakopoulou A, Konstantinidis A, Lappin D. Stem cell-like populations and immunoregulatory molecules in periodontal granulation tissue. *J Periodontal Res*, 2018; 53: 610-621.
8. Fawzy El-Sayed KM, Paris S, Becker ST, Neuschl M, De Buhr W, Sälzer S, Wulff A, Elrefai M, Darhous MS, El-Masry M, et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. *J Clin Periodontol*, 2012; 39:861–870.
9. Lee KE, Kang CM, Jeon M, Kim SO, Lee JH, Choi HJ. General gene expression patterns and stemness of the gingiva and dental pulp. *J Dent Sci*, 2022; 17(1):284-292.
10. Kim D, Lee AE, Xu O, Zhang Q, Le AD. Gingiva-Derived Mesenchymal Stem Cells: Potential Application in Tissue Engineering and Regenerative Medicine - A Comprehensive Review. *Front Immunol*, 2021; 16:12:667221.
11. Mohr A, Zwacka R. The future of mesenchymal stem cell-based therapeutic approaches for cancer—From cells to ghosts. *Cancer Lett*, 2018; 414: 239-249.
12. Zhang Q, Shi S, Liu Y, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol*, 2009; 183: 7787-7798
13. Górski B. Gingiva as a new and the most accessible source of mesenchymal stem cells from the oral cavity to be used in regenerative therapies. *Postepy Hig Med Dosw (Online)*, 2016; 17;70(0):858-71
14. Tomar GB, Srivastava RK, Gupta N, Barhanpurkar AP, Pote ST, Jhaveri HM, Mishra GC, Wani MR. Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine. *Biochem Biophys Res Commun*, 2010; 393(3):377-83.
15. Du L, Yang P, Ge S. Isolation and characterization of human gingiva-derived mesenchymal stem cells using limiting dilution method. *J Dent Sci.*,2016; 11(3):304-314.
16. Tomasello L, Mauceri R, Coppola A, Pitrone M, Pizzo G, Campisi G, Pizzolanti G, Giordano C. Mesenchymal stem cells derived from inflamed dental pulpal and gingival tissue: a potential application for bone formation. *Stem Cell Res Ther*, 2017; 1;8(1):179.
17. Liu X, Wang Z, Song W, Sun W, Hong R, Pot-hukuchi A, et al. Systematically transplanted human gingiva-derived mesenchymal stem cells regulate lipid metabolism and inflammation in hyperlipidemic mice with periodontitis. *Exp Ther Med*, 2020; 19:672–82.
18. Balaban YE, Akbaba S, Bozkurt SB, Buyuksungur A, Akgun EE, Gonen ZB, Salkin H, Tezcaner A, Hakki SS. Local application of gingiva-derived mesenchymal stem cells on experimental periodontitis in rats. *J Periodontol*, 2023.
19. Liu X, Wang Z, Song W, Sun W, Hong R, Pot-hukuchi A, et al. Systematically transplanted human gingiva-derived mesenchymal stem cells regulate lipid metabolism and inflammation in hyperlipidemic mice with periodontitis. *Exp Ther Med*, 2020; 19:672–82.
20. Sun W, Wang Z, Xu Q, Sun H. The treatment of systematically transplanted gingival mesenchymal stem cells in periodontitis in mice. *Exp Ther Med*, 2019; 17(3):2199-2205.
21. Fawzy El-Sayed KM, Mekhemar MK, Beck-Broichsitter BE, Bähr T, Hegab M, Receveur J, Hennewer C, Becker ST, Wiltfang J, Dörfer. Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with IL-1 α -hydrogel synthetic extracellular matrix. *J Clin Periodontol*,2015; 42(5):448-57.
22. <https://www.clinicaltrials.gov/>; [online]. [viewed: 08.03.2024].

Digital or conventional impressions, which one to choose?

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Introduction: Dental impression are a negative copy of the teeth and oral tissues, into which gypsum or other die material can be processed to create a positive model of the dental structures. Dentistry in our days generates new information every year and digital dentistry is becoming established and influential. Dentists should stay abreast of new technologies, some of the conventional materials and less time-consuming techniques remain widely used. It is important to ensure safely and effectively impressing the exact form of the oral tissues and provide optimal patient management. The accuracy of the impression is of paramount importance for the functionality and longevity of the prosthetic restoration. **Aim:** The aim of the study is to introduce and compare advantages and disadvantages of digital and conventional impressions. **Material and methods:** The study was done by researching and comparing different sources in publicly available scientific online databases (such as PubMed, Scopus, Science Direct). **Results and discussion:** There were significant differences among the digital and conventional impressions in the working time and processing steps. Patients and dentists stated that digital impressions were more comfortable than conventional techniques. **Conclusion:** Digital impressions resulted in a more time-efficient technique than conventional impressions. They are cheaper for the dentist after an initial big investment.

Key words: *advantages, disadvantages, impression materials, impressions, digital dentistry*

Introduction

Dental impression are a negative copy of the teeth and oral tissues, into which gypsum or other die material can be processed to create a positive model of the dental structures. Dentistry in our days generates new information every year and digital dentistry is becoming established and influential. It is important to ensure safely and effectively impressing the exact form of the oral tissues and provide optimal patient management.

The introduction of computer-aided design/computer aided manufacturing (CAD/CAM) technology in dentistry has resulted in more accurate manufacturing of prosthetic frameworks, and greater accuracy of dental restorations, and the technology has improved since the 1980s [1, 2]. These impressions are created using digital scanners that use lasers or optical technology to capture intricate details of the patient's teeth and surrounding tissues. The resulting digital model is incredibly precise, offering accuracy down to the micrometer level. This high level of precision eliminates the potential for human error and distortions that can

occur with traditional impression materials. The development strategy of CAD/CAM techniques included automating the production process and optimizing the quality of restorations by using new biocompatible materials, especially high-performance ceramics, such as zirconia and lithium disilicate [3]. Several reports have demonstrated the potential for accurate and precise restorations using CAD/CAM technology. The accuracy of the impression depends on the materials themselves [4, 5], impression tray types [6, 7], and impression techniques. Each step in the process introduces potential human and/or material error [8].

Impression materials can be classified according to their composition, setting reaction, and setting properties, but a commonly used system is based on the properties after the material has set. At present, the most popular types of impression materials for removable, fixed, and implant prosthodontics are irreversible hydrocolloids, polyethers and PVSs. Alginate impression materials are used for full-arch impressions, for impression of partial and complete removable

dental prosthesis. The hydrophilic nature of the material allows it to be used in the presence of saliva and blood with a moderate ability to reproduce details [9]. Polyethers were introduced in the late 1960s. These materials are hydrophilic, allowing them to be used in a moist environment. Their good wetting properties also allow gypsum casts to be made more easily. The chemical reaction for PVS involves a base paste containing hydrosilane-terminated molecules reacting with an accelerator paste containing siloxane oligomers with vinyl end groups and a platinum catalyst. Although there is no by-product formed, there is often a secondary reaction that can release hydrogen in the presence of hydroxyl groups, commonly found in impurities from the oligomerization reaction of the siloxane molecule [10].

Fast-set impression materials have been advocated to reduce chair time and facilitate the impression process, qualities that can be especially important when treating patients with severe gag reflexes [11].

Aim

The aim of the study is to introduce and compare advantages and disadvantages of digital and conventional impressions.

Material and methods

The study was done by researching and comparing different sources in publicly available scientific online databases (such as PubMed, Scopus, Science Direct). Keywords, that were used “advantages”, “disadvantages”, “impression materials”,

“impressions”, “digital dentistry”. Only articles in English were included.

Results and discussion

There were significant differences among the digital and conventional impressions in the working time and processing steps. Patients and dentists stated that digital impressions were more comfortable than conventional techniques. The advantages and disadvantages of digital impressions with respect to conventional physical impressions (i.e. impressions made with trays and materials) are presented below and summarised in Table 1.

The ability to directly capture all dental arch information of the patient, and consequently their 3D models, without using conventional physical impressions, is one of the advantages of digital impressions [12, 13]. In fact, the conventional physical impressions can cause momentary discomfort for the patient due to the inconvenience and hardship stemming from the materials positioned on impression trays (whether generic or individualised) [12, 14]. Some patients (e.g. patients with strong gag reflex, or children) appear not to tolerate the classic procedure [14]. For such patients, replacing conventional impression materials with light is an advantage; optical impression is therefore appreciated [15]. Digital impression decreases patient discomfort significantly when compared to traditional physical impression. In fact, it eliminates the need for materials and impression trays, which are often unwelcome to the patient [16, 17].

Table 1. Advantages and disadvantages of digital impressions and conventional impressions

	Conventional impressions	Digital impressions
Advantages	Profitability	Less patient discomfort
	Proven track record	Time – efficient
	Flexibility in materials	Simplified clinical procedures.
Disadvantages	Long time of working process	Difficulty detecting deep marginal lines of prepared teeth
	Discomfort of patient with gag reflex	Purchasing and managing cost
	Make a mistake in every stage of work	Learning curve

Several studies have shown that optical impressions are time-efficient, as they enable reduction of the working times (and therefore costs) when compared to conventional impressions. Despite the recent technological advancements in IOS, with the latest devices introduced in the market enabling the capture of a full-arch scan in less than 3 min, it does not appear that the major differences in time efficiency stem from the act of making an impression itself (a full-arch scan may take 3–5 min, similar to that required for conventional impressions), but rather from the time saved afterwards, during all subsequent steps [18]. In fact, with optical impressions, there is no need to pour stone casts and obtain physical plaster models. It is possible to e-mail the 3D virtual models (proprietary or STL files) of the patient directly to the dental laboratory without the need to deliver anything via courier or regular mail [19]. This enables the saving of a considerable amount of time and money during the working year [20]. Another benefit conferred by the use of optical impression is clinical. In fact, when the learning curve has been completed, the use of IOS may confer further clinical advantages, simplifying impression-making in complex cases, for example in the presence of multiple implants or severe undercuts that may render the detection of a conventional impression difficult and insidious [21]. Moreover, if the clinician is not satisfied with some of the details of the recorded optical impression, they may delete them and recapture the impression without having to repeat the entire procedure; this aspect is time-saving [22]. For the clinician, optical impression allows the skipping of an otherwise unavoidable step (the conventional impression is based on the detection of physical impressions and subsequent casting of gypsum models) with a time-saving effect. The elimination of conventional

impression materials translates into direct savings for the clinician, with reduced consumables costs [23]. With IOS, the clinician and the dental technician can assess the quality of the impression in real-time. In fact, immediately after the scan has been performed, the dentist can e-mail it to the laboratory, and the technician can check it accurately. If the dental technician is not convinced of the quality of the received optical impression, he/she can immediately request that the clinician make another one without any loss of time and without having to call the patient for a second appointment. This aspect simplifies and strengthens communication between the dentist and dental technician [24]. There is a learning curve for adopting IOS in the dental clinic, and this aspect must be considered with attention. Subjects with a greater affinity for the world of technology and computers (e.g. young dentists) will find it very easy to adopt IOS in their practice [25]. Lastly, it should be kept in mind that it is still unclear whether one scanning strategy is better than the other, as manufacturers provide little information about their scanning strategies. One of the most frequent problems encountered with IOS and with optical impressions is difficulty in detecting deep marginal lines on prepared teeth or in the case of bleeding. In some cases, in fact, and especially in aesthetic areas where it is important for the clinician to place the prosthetic margins subgingivally, it may be more difficult for the light to correctly detect the entire finishing line. In fact, unlike the conventional impression materials, light cannot physically detach the gum and therefore cannot register ‘non-visible’ areas. Similar problems can also occur in the event of bleeding, as blood may obscure the prosthetic margins [26]. Despite this, with the proper attention and speed (the gingival sulcus tends to close immediately after the

retraction cord is removed) and the appropriate strategies for highlighting the preparation line (insertion of a single or double retraction cord), and avoiding bleeding (excellent oral hygiene and provisionals with correct emergency profile), it is possible for the clinician to detect a good optical impression even in difficult contexts. Recently, some authors have suggested combining strategies, i.e. partly using conventional impression materials. Beyond that, a good optical impression is the result of many factors, namely the quality of prosthetic preparation, the patient's compliance with oral hygiene, and the goodness of the provisional restorations; as with conventional impressions, healthy soft tissues are essential for a good optical impression [27]. Over the last few years, manufacturers have released many new models on the market, and the growth in supply should be accompanied by a reduction in purchase costs. Regardless, the purchase cost of a high-end, last-generation IOS should be cushioned over the year by integrating the device into the clinical workflow across the various dental disciplines (prosthodontics, orthodontics, implant surgery). One important aspect to consider is additional managing costs related to upgrades of the reconstruction software. Different manufacturing companies have different policies in this regard, and it is important for the clinician to be fully informed of the annual management costs and fees, where present, before purchasing an IOS [28].

Conclusion

Both methods offer distinct advantages and limitations that cater to various practice needs, patient preferences, and financial considerations. Digital impressions resulted in a more time-efficient technique than conventional impressions. They are cheaper for the dentist after an initial big investment. In the end, the goal remains constant: to

provide patients with the highest quality of care, accurate diagnoses, and optimal treatment outcomes. By carefully considering the advantages and disadvantages of both impression techniques, dental professionals empower themselves to make informed decisions that align with their practice's goals and their patients' needs.

References

1. De La Cruz, JE, Funkenbusch PD, Ercoli C, Moss ME, Graser GN, Tallents RH. Verification jig for implant supported prosthesis: a comparison of standard impressions with verification jigs made of different materials. *J Prosthet Dent*. 2002; 88:329–336.
2. Mormann WH, Brandestini M, Lutz F. The Cerec system: computer-assisted preparation of direct ceramic inlays in 1 setting. *Quintessenz*. 1987; 38:457–470
3. Luthardt R, Weber A, Rudolph H, Schone C, Quaas S, Walter M. Design and production of dental prosthetic restorations: basic research on dental CAD/CAM technology. *Int J Comput Dent*. 2002;5:165–176.
4. Herbst D, Nel JC, Driessen CH, Becker PJ. Evaluation of impression accuracy for osseointegrated implant supported superstructures. *J Prosthet Dent*. 2000; 83(5):555–561.
5. Wee AG. Comparison of impression materials for direct multi-implant impressions. *J Prosthet Dent*. 2000; 83(3):323–331.
6. Brosky ME, Pesun IJ, Lowder PD, Delong R, Hodges JS. Laser digitization of casts to determine the effect of tray selection and cast formation technique on accuracy. *J Prosthet Dent*. 2002; 87(2):204–209.
7. Ceyhan JA, Johnson GH, Lepe X. The effect of tray selection, viscosity of impression material, and sequence of pour on the accuracy of dies made from dual-arch impressions. *J Prosthet Dent*. 2003; 90(2):143–149.
8. Rudd RW, Rudd KD. A review of 243 errors possible during the fabrication of a removable partial denture: part II. *J Prosthet Dent*. 2001; 86(3):262–276.
9. Reitz CD, Clark NP. The setting of vinyl polysiloxane and condensation silicone putties when mixed with gloved hands. *J Am Dent Assoc* (1988)
10. Petrie CS, Walker MP, O'Mahony AM, Spencer P. Dimensional accuracy and surface detail reproduction of two hydrophilic vinyl polysiloxane impression materials tested under dry, moist, and wet conditions. *J Prosthet Dent* (2003)
11. Wadhvani CP, Johnson GH, Lepe X, Raigrodski AJ. Accuracy of newly formulated fast-setting elastomeric impression materials. *J Prosthet Dent* (2005)

12. Ahlholm P, Sipilä K, Vallittu P, Jakonen M, Kotiranta U. Digital Versus Conventional Impressions in Fixed Prosthodontics: A Review. *J Prosthodont* 2016 Aug 2.
13. Chochlidakis KM, Papaspyridakos P, Geminiani A, Chen CJ, Feng IJ, Ercoli C. Digital versus conventional impressions for fixed prosthodontics: A systematic review and meta-analysis. *J Prosthet Dent*. 2016; 116 (2): 184–190.e12.
14. Muir JD, Calvert EJ. Vomiting during the taking of dental impressions. Two case reports of the use of psychological techniques. *Br Dent J*. 1988; 165(4):139–141.
15. Means CR, Flenniken IE. Gagging – a problem in prosthetic dentistry. *J Prosthet Dent*. 1970; 23(6):614–620.
16. Yuzbasioglu E, Kurt H, Turunc R, Bilir H. Comparison of digital and conventional impression techniques: evaluation of patients' perception, treatment comfort, effectiveness and clinical outcomes. *BMC Oral Health*. 2014; 14:10.
17. Christensen GJ GJ. Will digital impressions eliminate the current problems with conventional impressions? *J Am Dent Assoc*. 2008; 139(6):761–763.
18. Grünheid T, McCarthy SD, Larson BE. Clinical use of a direct chairside oral scanner: an assessment of accuracy, time, and patient acceptance. *Am J Orthod Dentofac Orthop*. 2014; 146(5):673–682.
19. Schepke U, Meijer HJ, Kerdijk W, Cune MS. Digital versus analog complete arch impressions for single-unit premolar implant crowns: Operating time and patient preference. *J Prosthet Dent*. 2015; 114(3):403–406.
20. Burhardt L, Livas C, Kerdijk W, van der Meer WJ, Ren Y. Treatment comfort, time perception, and preference for conventional and digital impression techniques: A comparative study in young patients. *Am J Orthod Dentofac Orthop*. 2016; 150(2):261–267.
21. Park HR, Park JM, Chun YS, Lee KN, Kim M. Changes in views on digital intraoral scanners among dental hygienists after training in digital impression taking. *BMC Oral Health*. 2015; 15(1):151.
22. Kim J, Park JM, Kim M, Heo SJ, Shin IH, Kim M. Comparison of experience curves between two 3-dimensional intraoral scanners. *J Prosthet Dent*. 2016; 116(2):221–230.
23. Joda T, Bragger U. Digital vs. conventional implant prosthetic workflows: a cost/time analysis. *Clin Oral Implants Res*. 2015; 26(12):1430–1435.
24. Mangano F, Gandolfi A, Luongo G. Intraoral scanners in dentistry: a review of the current literature. *BMC Oral Health*. 2017 Dec 12
25. Agnini A, Agnini A, Coachman C. *The Digital Revolution: The Learning Curve*. 1st edition, Quintessence Publishing, 2015.
26. Lim JH, Park JM, Kim M, Heo SJ, Myung JY. Comparison of digital intraoral scanner reproducibility and image trueness considering repetitive experience. *J Prosthet Dent*. 2017 Jul 7.
27. Mandelli F, Ferrini F, Gastaldi G, Gherlone E, Ferrari M. Improvement of a Digital Impression with Conventional Materials: Overcoming Intraoral Scanner Limitations. *Int J Prosthodont*. 2017; 30(4):373–376.
28. Zimmermann M, Mehl A, Mörmann WH, Reich S. Intraoral scanning systems - a current overview. *Int J Comput Dent*. 2015; 18(2):101–129.

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