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Letter from the Editor-in-Chief
Medical Research Journal
University of Belgrade, Faculty of Medicine

Dear Readers, Researchers, and Authors,

It is my pleasure to present the current issue of *Medical Research*.

With this issue, we are introducing a new section, Editorial, which appears in our journal for the first time. We believe that editorials will provide a valuable critical review of this issue's content relevant to the medical and biomedical research community. We hope this addition will further enrich our journal's content and stimulate academic discussion among our readers.

The articles published in this issue reflect our continued commitment to advancing scientific knowledge through high-quality, peer-reviewed research. We are grateful to our authors, reviewers, and editorial board members whose dedication and expertise make the publication of each issue possible.

As our journal continues to grow, we encourage researchers and authors to engage with the scientific contributions published in *Medical Research*. We kindly invite authors

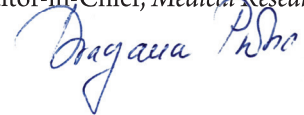
to consider relevant articles from our journal when conducting literature reviews and preparing manuscripts. Appropriate citation of previously published work not only acknowledges the contributions of fellow researchers but also strengthens scientific dialogue and enhances the visibility and impact of the valuable research disseminated through our journal.

We welcome new submissions and encourage scientists from diverse disciplines within medicine and health sciences to share their findings with the international research community through *Medical Research*.

Thank you for your continued support and trust in our journal. We hope you find this issue informative, inspiring, and valuable for your future research endeavors.

Sincerely,

Prof. Dragana Protic, MD, PhD
Editor-in-Chief, *Medical Research*

A handwritten signature in blue ink that reads "Dragana Protic". The signature is written in a cursive, flowing style.

EDITORIAL

Editorial for the second issue of volume 59 of the medical journal “Medical Research”

Nenad Vasic 

Clinic Center Christophsbad, Clinic for Psychiatry and Psychotherapy, Göppingen, Germany

Dear colleagues and friends, it is a great pleasure to summarize the articles of the second issue of volume 59 of the medical journal “Medical Research” published by the Faculty of Medicine, University of Belgrade.

In this issue of **Medical Research**, several researchers report on current, relevant topics – particularly in internal medicine, cardiology, radiology, and oncology – mostly through interesting small-scale studies and reviews.

Pavic, S. et al. report on a recent case of a snakebite in Western Serbia with a fatal outcome, present current treatment recommendations, especially highlighting the need for extensive and prolonged monitoring following such a bite (1). *Mehmedbegovic, Z. et al.* investigated the methodology for measuring the lumen diameter of the left main coronary artery, demonstrating that optical coherence tomography (OCT) is more suitable to capture outliers than quantitative coronary analysis (QCA) (2). In her review, *Bozic, M.* identified some critical issues in the diagnosis and treatment of glaucoma. In particular, misconceptions can occur when different methods and evaluations during measuring intraocular pressure are applied (3). The research group led by *M. Buta* studied the development of distant metastasis following breast cancer, monitoring and examining patients over an extended period of time. Their results demonstrate that adequate surgical resection remains of critical importance (4). The study by *Nedeljkovic-Arsenovic, O. et al.* was conducted at the University Clinical Centre of Serbia, Center of Ra-

diology, and focuses on the diagnostics of hypertrophic cardiomyopathy (HCM). It reveals the cardiac magnetic resonance (CMR) as the gold standard among non-invasive methods (5). *Protic, D. et al.* summarize existing studies on fragile X syndrome and its pharmacological treatment options, specifically addressing defects in the signalling pathway caused by the *FMR1* gene. The authors discuss gaps in the availability of pharmacological support and outline potential directions for further molecular biology research (6). *Markovic, N. et al.* conducted an *in silico* study (computer based molecular docking simulations to predict interactions between ligands and receptors), demonstrating the beneficial effects of the Mediterranean diet on key proteins in the osteoclast differentiation signaling pathway, which are due to its high content of some bioactive compounds. Essentially, they showed the inhibitory effect of these compounds on the Akt protein (protein kinase B) and, consequently, on osteoclast activity (7). The paper by *Otasevic, P. et al.* summarizes the current state of knowledge regarding the role of iron deficiency (ID) and subsequently iron supplementation in patients with heart failure with reduced ejection fraction, suggesting the reduced mortality following the treatment (8).

We invite you to learn more about these interesting and relevant topics, and to understand the published works not only as a theoretical foundation but also as a source of practical knowledge, which you can apply in practice. We hope you enjoy reading it.

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ORIGINAL ARTICLE

The effect of the most abundant bioactive compounds of the Mediterranean diet on the osteoblast-mediated osteoclast differentiation signaling pathway: *in silico* analysis

✉ Nemanja Markovic^{ID1}, Svetozar Mijuskovic^{ID1}, Mirjana Jovanovic^{ID2}, Nikola Mitovic^{ID2}, Aleksandra Nenadovic^{ID2}, Andrija Vukovic^{ID2}, Sanjin Kovacevic^{ID2}, Marija Kosic^{ID3}, Jovan Juloski^{ID4,5}, Marija S. Stankovic^{ID2}, Jelena Nesovic Ostojic^{ID2}

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Summary

Introduction: Bones are fundamental components of the skeletal system that undergo continuous remodeling throughout life. Osteoclasts are responsible for bone resorption, while osteoblasts form new bone matrix and indirectly influence osteoclast differentiation through signaling pathways involving molecules such as M-CSF, c-Fms, GRB2, ERK, PI3K, and Akt. To maintain bone health, increasing attention is being given to nutritional interventions, including the Mediterranean diet, which is rich in bioactive compounds. The aim of this study was to perform an *in silico* analysis of potential interactions between the most prevalent bioactive compounds of the Mediterranean diet and the key proteins of the osteoclast differentiation signaling pathway.

Materials and Methods: A library comprising 115 predominant bioactive compounds from the Mediterranean diet was constructed based on literature data. Protein structures obtained via crystallography were used as targets in molecular docking simulations to predict interactions between ligands and receptors.

Results: Most ligands did not show significant interactions with the investigated proteins ($\Delta G > -7$ kcal/mol), except in the case of Akt protein. For this protein, a larger number of ligands demonstrated ΔG values < -7 kcal/mol. The five most potent ligands (salvianolic acid, apigenin, diosmin, mangiferin and sylbin) bound to amino acid sequences forming the active site of the receptor, indicating a potential inhibitory effect.

Conclusion: The *in silico* analysis suggests that bioactive components of the Mediterranean diet possess potential to inhibit Akt protein (protein kinase B), thereby opening new perspectives for their application in modulating osteoclast activity and developing novel strategies for maintaining bone health.

Keywords: Mediterranean diet; bioactive compounds; osteoblasts; osteoclasts; protein kinase B

Cite this article as: Markovic N, Mijuskovic S, Jovanovic M, Mitovic N, Nenadovic A, Vukovic A, Kovacevic S, Kosic M, Juloski J, S. Stankovic M, Nesovic Ostojic J. The effect of the most abundant bioactive compounds of the mediterranean diet on the osteoblast-mediated osteoclast differentiation signaling pathway: in silico analysis; Medicinska istraživanja 2026; 59(2):75-82; DOI: 10.5937/medi0-60144

INTRODUCTION

The skeletal system forms the structural framework of the body, which, in functional terms, together with the muscular system, enables movement. Bones, as the principal and most abundant anatomical components of the skeletal system, are continuously remodeled and adapt to mechanical loads that the body endures. This process depends on a delicate balance between the activities of osteoclasts and osteoblasts. In this process, osteoclasts perform bone resorption, while osteoblasts create new bone matrix and directly influence osteoclast differentiation (1–3).

One of the signaling pathways responsible for osteoclast differentiation, orchestrated by osteoblasts, begins with the binding of M-CSF (macrophage colony-stimulating factor) to the receptor with a tyrosine kinase domain, c-Fms (colony-stimulating factor 1 receptor), on the surface of osteoclast precursor cells. As a result of this activation, autophosphorylation occurs on specific tyrosine residues in the intracellular part of the receptor, after which the pathway divides into two branches (4–5). In one branch, phosphorylated c-Fms leads to the recruitment and activation of the adapter protein GRB2 (growth factor receptor-bound protein 2). Activated GRB2 further triggers a cascade of (de)phosphorylation mediated by MAP (mitogen-activated protein) kinases, ultimately activating ERK (extracellular signal-regulated kinase). Phosphorylated ERK enters the nucleus of precursor cells and regulates the expression of genes crucial for the proliferation and survival of osteoclast precursors.

In the other branch, phosphorylated c-Fms activates PI3K (phosphoinositide 3-kinase), which catalyzes the addition of a phosphate group to phosphatidylinositol-4,5-bisphosphate (PIP₂), converting it into phosphatidylinositol-3,4,5-trisphosphate (PIP₃). PIP₃ enables the translocation of protein kinase B (Akt) to the membrane, where it is phosphorylated and activated by PDK1 (phosphoinositide-dependent kinase 1). Activated protein kinase B inhibits pro-apoptotic proteins such as Bax

and FoxO, thereby promoting cell survival. It also leads to cytoskeletal reorganization, which is necessary for the motility of osteoclast precursors and prepares them for fusion and formation of mature osteoclasts (Figure 1).

Excessive activation of these signaling pathways can lead to increased bone resorption. Increased osteoclast activity can occur in various conditions such as growth, fractures, osteoporosis, and others. In osteoporosis, an imbalance arises between bone resorption and new bone formation, resulting in reduced bone density, making bones more prone to fractures (6–7).

In order to stimulate bone health and correct various osteological pathological conditions, different interventions are undertaken, among which nutritional approaches are increasingly highlighted. One such intervention is the introduction of the Mediterranean diet, which has shown numerous positive effects on bone health (8–10). The Mediterranean diet is based on the traditional habits of the Mediterranean region's population and consists of a high intake of fruits, vegetables, whole grains, olive oil, nuts, legumes, and seeds (10, 11). The consumption of red meat, processed foods, and sugars is limited to a few times per month, while fish and seafood are consumed regularly. Moderate consumption of dairy products and red wine is also recommended (11). The Mediterranean diet is rich in calcium and vitamin D, which are crucial for bone mineralization and maintenance of bone density (12). Components from olive oil and fish rich in omega-3 fatty acids support the balance between osteoclasts and osteoblasts, thereby reducing bone loss (13). The Mediterranean diet also helps maintain a healthy body weight (14–16), which reduces mechanical stress on bones and further contributes to preserving bone mass. Combining this diet with physical activity and adequate sun exposure for vitamin D synthesis further improves bone health.

The aim of our study was to investigate, using an *in silico* approach, the interactions of the most abundant bioactive compounds of the Mediterranean diet with the mentioned components of the signaling pathway responsible for osteoblast-mediated osteoclast differentiation.

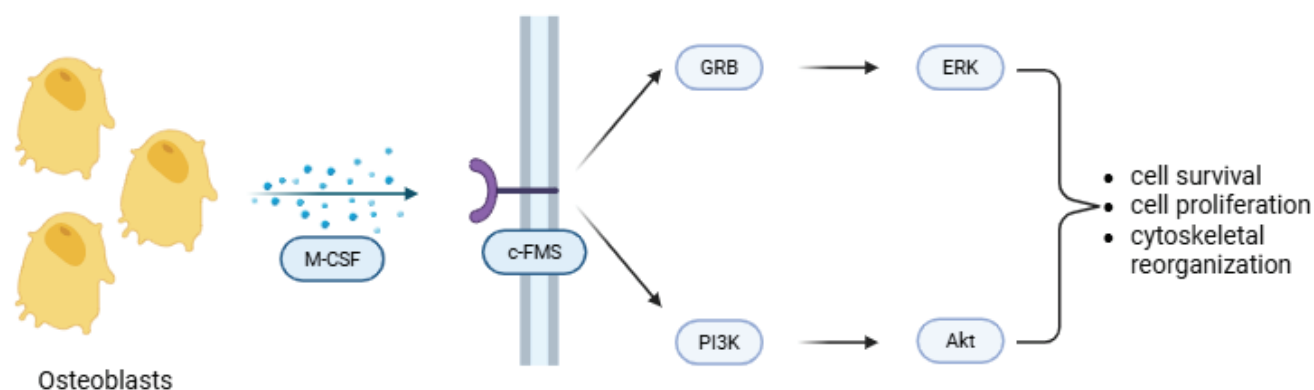


Figure 1. The signaling pathway responsible for osteoblast-mediated osteoclast differentiation.

MATERIALS AND METHODS

Ligand Library Definition

PubMed database search using the parameters “Mediterranean diet” AND “bioactive compounds” identified a total of 217 articles. Articles focusing on the chemical identification of individual molecules present in foods predominantly included in the Mediterranean diet were analyzed. Based on the data from these studies, a ligand library containing 115 bioactive compounds was established, which we further grouped according to their food sources.

Preparation for Molecular Docking

The crystallographic structures of the proteins (receptors) M-CSF, c-Fms, GRB2, ERK, PI3K, and protein kinase B (Akt) were obtained from the PDB or AlphaFold databases. The 3D structures of all ligands were retrieved from PubChem (17).

Molecular Docking

Molecular docking is a technique used to predict the orientation, affinity, and interaction of a ligand at the binding site of a receptor (protein). In this study, we employed the AutoDock Vina software, which is widely used for predicting these interactions and has found extensive application in drug discovery (18). Upon launching the software, the protein structures (in PDB format) and the ligand library (in SDF format) were imported and modified as needed. For analyses, water molecules were removed from the examined protein structures. In most cases, water molecules do not play a significant role in binding and are therefore deleted to facilitate calculations. Additionally, polar hydrogen atoms and charges were added to the receptor molecules before initiating the process, in order to “open” as many binding sites as possible. Molecular docking was performed under conditions where all ligand bonds were set as rotatable, while receptor bonds were fixed (18). After initiating the docking, the program simulates interactions between different functional groups of the receptors and ligands. Ultimately, the ten most representative interactions are displayed, along with their Gibbs free energy values and root mean square deviation (RMSD) of atomic positions. More negative Gibbs free energy values indicate a higher likelihood of spontaneous reaction and stronger binding between receptor and ligand, while RMSD values are used to evaluate the precision of the performed docking (18).

Characterization of Ligand–Receptor Interactions

To determine the nature of the interactions between the examined ligands and receptors, the PyMOL software

was used for those interactions with Gibbs free energy values below -7 kcal/mol. All obtained interactions were imported into the software, and the amino acid sequences, specifically the pockets where ligands were bound were analyzed. If the amino acid sequence corresponded to the active site or the domain responsible for the protein’s biological activity, the interaction was considered inhibitory.

RESULTS

A total of 115 compounds were identified based on literature searches focusing on bioactive compounds from the Mediterranean diet.

The AutoDock Vina program was used for molecular docking between the ligand library and the receptors M-CSF, c-Fms, GRB2, ERK, PI3K, and protein kinase B (Akt). As an empirical threshold for higher spontaneous binding probability, interactions with Gibbs free energy values below -7 kcal/mol were considered.

The interaction between M-CSF and diosgenin showed the lowest Gibbs free energy value at -3.23 kcal/mol, while sulforaphane exhibited the highest value at -0.079 kcal/mol (**Figure 2A**).

For c-Fms, the interaction with ursolic acid showed the lowest Gibbs free energy value at -3.9 kcal/mol, while sulforaphane had the highest value at -0.993 kcal/mol (**Figure 2B**).

Regarding GRB2, the interaction with diosgenin had the lowest Gibbs free energy value at -3.82 kcal/mol, and sulforaphane the highest at -1.1 kcal/mol (**Figure 2C**).

The interaction between ERK and diosgenin showed the lowest Gibbs free energy value at -3.71 kcal/mol, **whereas tannic acid had the highest value at -1 kcal/mol** (**Figure 2D**).

For PI3K, diosgenin again showed the lowest Gibbs free energy value at -3.75 kcal/mol, while tannic acid had the highest at -0.95 kcal/mol (**Figure 2E**).

The interaction between protein kinase B (Akt) and salvianolic acid showed the lowest Gibbs free energy value at -9.47 kcal/mol, while sulforaphane had the highest value at -3.36 kcal/mol (**Figure 2F**).

Based on these results, the binding of the 5 most potent ligands was further analyzed to determine the nature of their interactions. Five top ligands were selected for additional investigation: salvianolic acid, apigenin, diosmin, mangiferin, and silybin. Salvianolic acid primarily interacted with amino acids LYS161, GLY160, PHE159, THR158, GLY157, and LYS156, which were also common binding sites for other ligands, particularly diosmin and mangiferin. This pattern indicates that regions rich in lysine and glycine residues are critical for binding stability. Apigenin partially shared binding sites with salvianolic acid, especially at LYS161, GLY160, and LYS156, but additionally bound to HIS236, PHE235,

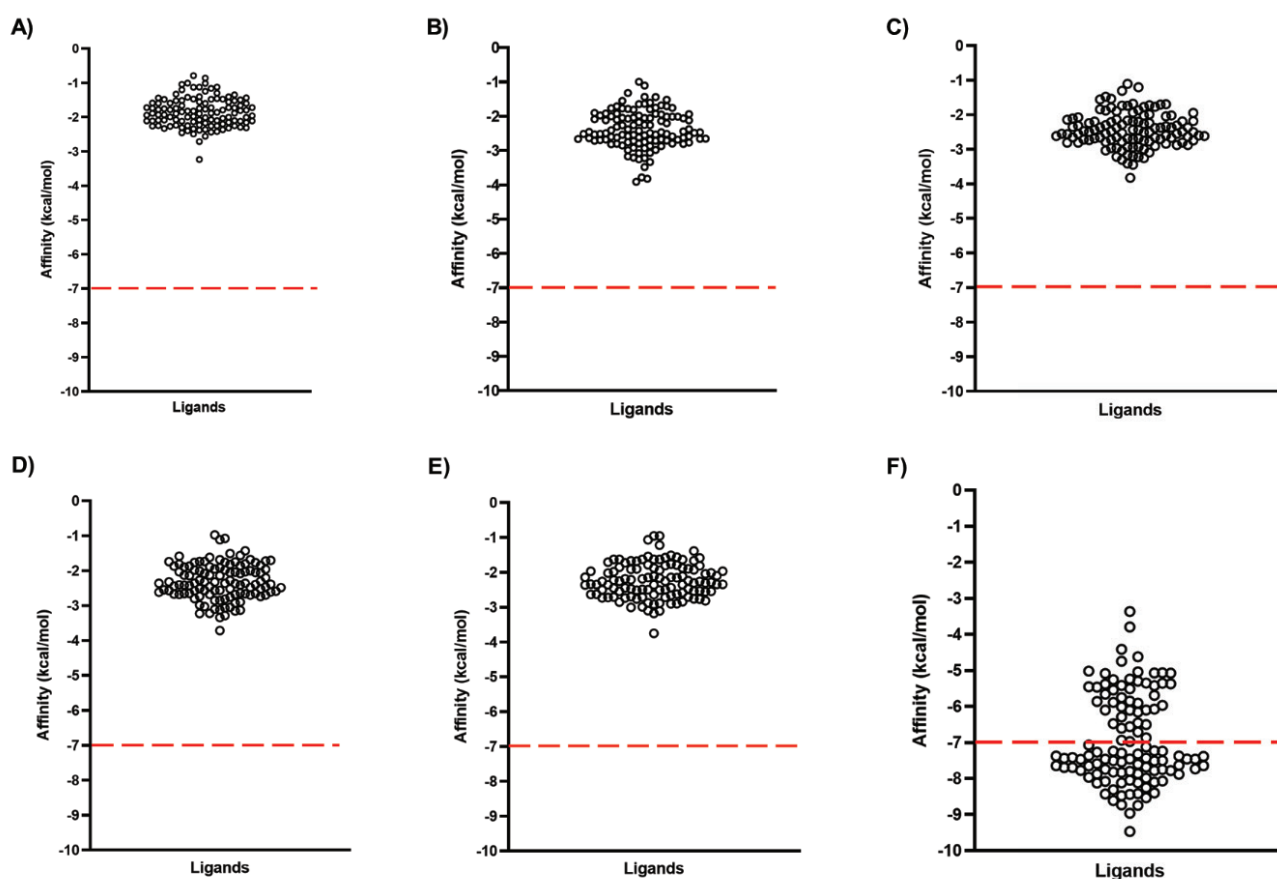


Figure 2. Gibbs free energy values for interactions between the tested ligand library and each of the investigated proteins: (A) M-CSF, (B) c-FMS, (C) GRB2, (D) ERK, (E) PI3K and (F) Akt. Each point represents an individual ligand from the Mediterranean diet-derived library evaluated by molecular docking. The red dashed line indicates the empirical threshold ($\Delta G = -7$ kcal/mol), below which interactions are considered to have higher spontaneity and potential biological significance. Notably, only interactions with Akt (F) demonstrated multiple ligands surpassing this threshold, suggesting a potential inhibitory effect.

PHE234, and LEU233, suggesting additional interactions in a different region of the protein, near aromatic residues. Diosmin exhibited the most comprehensive spectrum of interactions, covering nearly all amino acids involved in binding with salvianolic acid and mangiferin (the GLY155–LYS161 sequence), as well as additional residues such as ASP280, ASP415, LYS416, and LYS417, indicating potentially stronger and broader binding across various functional domains of Akt. Mangiferin shared the identical binding sequence from GLY155 to LYS161 with diosmin and salvianolic acid, suggesting a similar binding mechanism in the conserved region of the protein. However, it also showed additional interactions with MET225, GLY226, and TYR227, which were not observed for salvianolic acid. Silybin significantly differed from the other ligands, primarily binding to a completely different set of amino acids (PRO50, TYR49, PRO48, LEU47, ASP46) and additionally to regions such as LEU359–ILE363 and LEU380–ILE382, which were not involved with other ligands. This suggests that silybin targets a distinct functional domain of Akt (Table 1).

Finally, by filtering all amino acids interacting with these ligands, specific sequences of the Akt protein responsible for its biological activity and regulation were analyzed. The P-loop region (positions 156–162, sequence

KLGGFGK) represents a conserved motif crucial for ATP binding and kinase activity regulation. Salvianolic acid, apigenin, diosmin, and mangiferin all showed binding to this region. The HRD motif (positions 194–196, sequence HRD) is important for kinase catalytic activity; however, none of the analyzed ligands exhibited binding to this motif. The DFG motif (positions 292–294, sequence DFG) plays a key role in maintaining the active conformation of the kinase. Diosmin and mangiferin interacted with this region. The activation segment (positions 307–311, sequence CGLKE) is involved in modulating the conformation required for protein activity. Only salvianolic acid bound to this region. The regulatory region (positions 174–181, sequence YAMKILKK) controls the accessibility of the activation segment and overall protein stability. Salvianolic acid also demonstrated binding to this region (Table 2).

DISCUSSION

Various studies have demonstrated the positive effects of the Mediterranean diet on human health, as well as its role in preventing different diseases (19–21). Our research focused on the impact of the most abundant

Table 1. Amino acid sequences in protein kinase B (Akt) that interacted with the most potent ligands.

Ligands	Amino acids involved in binding
Salvianolic acid	LYS161, GLY160, PHE159, THR158, GLY157, LYS156, LYS187, ASP188, GLU189, MET278, LEU277, ASN276, ILE287, LYS286, THR218, THR217, GLN216, PHE215, ALA314, LEU313, TYR312, GLU311, CYS307, GLY308, LYS177, MET176, ALA175, TYR174
Apigenin	HIS236, PHE235, PHE234, LEU233, GLU232, GLY231, PRO421, PRO420, VAL419, LEU418, LEU277, MET278, LEU279, LYS156, GLY155, LYS161, GLY160, TYR277, VAL228, GLN411, ASP412
Diosmin	GLY155, LYS156, GLY157, THR158, PHE159, GLY160, LYS161, TYR227, VAL228, ASP280, LEU279, ASP415, LYS416, LYS417, LEU418, VAL419, PRO420, ILE285, LYS286, ILE287, THR288, ASP289, PHE290, GLY291, LEU292, TYR261, ASN276, TYR251, GLY252, ALA253, GLU254, GLU275, LEU274, ILE255, VAL256, SER257, ALA258, LEU259, ASP260, LEU261
Mangiferin	GLY155, LYS156, GLY157, THR158, PHE159, GLY160, LYS161, MET225, GLV226, TYR227, LEU211, SER210, THR209, THR288, ASP289, VAL190, LEU200
Sylbin	PRO50, TYR49, PRO48, LEU47, ASP46, LEU359, MET360, GLU361, ASP362, ILE363, ASP384, LYS383, ILE382, LEU381, LEU380, GLU316, PRO315, PRO345, LEU344, ARG343, GLY342, CYS341

Abbreviations: LYS – lysine, GLY – glycine, PHE – phenylalanine, THR – threonine, ASP – aspartic acid, GLU – glutamic acid, MET – methionine, LEU – leucine, ASN – asparagine, ILE – isoleucine, GLN – glutamine, ALA – alanine, TYR – tyrosine, CYS – cysteine, HIS – histidine, PRO – proline, VAL – valine, ARG – arginine.

Table 2. Overview of the regions, sequences, and positions of amino acids that regulate and enable the biological activity of protein kinase B (Akt), and the most potent ligands binding to them.

Region	Sequence	Position	Ligands
P-loop	KLGGFGK	156 – 162	salvianolic acid, apigenin, diosmin, mangiferin
HRD motif	HRD	194 – 196	/
DFG motif	DFG	292 – 294	Diosmin, mangiferin
Activation segment	CGLKE	307 – 311	salvianolic acid
Regulatory region	YAMKILKK	174 – 181	salvianolic acid

Abbreviations: K – lysine; L – leucine; G – glycine; F – phenylalanine; H – histidine; R – arginine; D – aspartic acid; C – cysteine; E – glutamic acid; Y – tyrosine; A – alanine; M – methionine; I – isoleucine.

bioactive molecules from the Mediterranean diet on a specific osteoblast-mediated signaling pathway responsible for osteoclast differentiation, which is crucial for bone function and homeostasis. The Mediterranean diet has shown numerous benefits in preventing fractures and osteoporosis. Adherence to this dietary pattern in non-Mediterranean regions has been associated with higher bone mineral density and a reduced risk of fractures, particularly in postmenopausal women (22–23). Treatment and prevention of osteoporosis begin with a healthy lifestyle and proper nutrition (24). A prospective observational study by Quattrini et al. demonstrated that perimenopausal and postmenopausal women adhering to the Mediterranean diet had a significantly increased dietary calcium intake, essential for maintaining bone health (25).

Based on our results, the highest Gibbs free energy values of ligand–receptor interactions were observed for sulforaphane and tannic acid, while the lowest were for diosgenin and salvianolic acid. Salvianolic acid showed the lowest Gibbs free energy value when interacting with protein kinase B (Akt), at -9.47 kcal/mol. An experimental study by Cao et al. confirmed a significant role of salvianolic acid in promoting fracture healing by balancing osteoblast and osteoclast differentiation. They demonstrated

that salvianolic acid dose-dependently inhibited osteoclast differentiation of bone marrow-derived macrophages, associated with NF- κ B suppression. Thus, in addition to interacting significantly with protein kinase B, salvianolic acid also acts on NF- κ B, thereby promoting bone fracture healing (26). Salvianolic acid also induces apoptosis and suppresses tumor growth in acute myeloid leukemia by inhibiting protein kinase B, as shown in an experimental study in mice by Pei et al. (27). Moreover, Yan et al. demonstrated that salvianolic acid alleviates inflammation and prevents pathological fibrosis by inhibiting CD36-mediated PI3K-Akt pathway activation in synovial fibroblasts isolated from human shoulder biopsies (28). Based on these studies, we can conclude that there is significant interaction between salvianolic acid and protein kinase B.

Although sulforaphane did not show significant interactions with any protein in our study, an experimental study by Kim et al. demonstrated its role in inhibiting osteoclastogenesis by acting on NF- κ B. They found that sulforaphane selectively inhibits NF- κ B. Likewise, their study did not confirm significant interaction with M-CSF and c-Fms proteins, as was also the case in our study, suggesting that the inhibitory effect on osteoclastogenesis is not mediated through interaction with these proteins (29).

Tannic acid also did not show significant interactions in our study; however, despite the absence of significant nutritional effects, it plays an important role in orthopedic surgery, as shown in the study by Sun et al. Tannic acid is a natural polyphenolic compound, and polyphenol-based materials play a major role in orthopedic transplantation. This compound can form strong interactions with metals, creating stable coatings on their surfaces, thereby improving the physical and chemical properties of bone implant surfaces and increasing implantation success rates (30).

Additionally, diosgenin did not show significant interactions with most of the examined receptor in our study, yet it is considered to play a significant role in bone formation. This was confirmed by an experimental study by Alper et al., which investigated the therapeutic effects of diosgenin on alveolar bone loss and apoptosis in diabetic rats with experimental periodontitis. The study revealed that diosgenin significantly improved bone formation and contributed to periodontal healing by increasing the expression of BMP-2 (bone morphogenetic protein 2), alkaline phosphatase, osteocalcin, and type I collagen, while reducing apoptosis and RANKL expression (31).

The most significant ligand interactions were observed with protein kinase B. Besides salvianolic acid, the most potent ligands binding to the active site of protein kinase B included apigenin, diosmin, and mangiferin. The inhibitory effect of apigenin on protein kinase B was confirmed in a study by Yang et al., who demonstrated that apigenin induces apoptosis in hepatocellular carcinoma cells by inhibiting the PI3K/AKT/mTOR signaling pathway (32). Mangiferin also has a significant im-

pact on protein kinase B, as evidenced in a study by Shi et al., which investigated the inhibitory effect of mangiferin on cell migration and angiogenesis through PI3K/AKT/mTOR signaling (33).

CONCLUSION

Our results did not demonstrate significant interactions between the investigated ligands and receptors, except in the case of protein kinase B (Akt). During the interaction of protein kinase B with the five most potent ligands examined further, it was determined that salvianolic acid, apigenin, diosmin, and mangiferin bind to amino acid sequences corresponding to the active site of the receptor, and this interaction is therefore considered inhibitory.

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UTICAJ NAJZASTUPLJENIJIH BIOAKTIVNIH JEDINJENJA MEDITERANSKE ISHRANE NA SIGNALNI PUT OSTEOLASTIMA POSREDOVANE DIFERENCIJACIJE OSTEOKLASTA: *IN SILICO* ANALIZA

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Sažetak

Uvod: Kostiju su osnovni elementi skeletnog sistema koji se neprekidno remodeluju. Osteoklasti vrše resorpciju kosti, dok osteoblasti formiraju novi koštani matriks i posredno utiču na diferencijaciju osteoklasta putem signalnih puteva u kojima učestvuju signalni molekuli: M-CSF, c-Fms, GRB2, ERK, PI3K i Akt. U cilju očuvanja zdravlja kostiju danas se sve značajnija pažnja posvećuje nutritivnim intervencijama, poput mediteranske dijeta koja je bogata bioaktivnim jedinjenjima. Cilj ovog rada je *in silico* ispitivanje potencijalnih interakcija najzastupljenijih bioaktivnih jedinjenja mediteranske dijeta sa navedenim proteinima signalnog puta diferencijacije osteoklasta.

Materijali i metode: Formirana je biblioteka od 115 najzastupljenijih bioaktivnih jedinjenja mediteranske dijeta identifikovanih iz literaturnih podataka. Strukture proteina dobijene kristalografijom korišćene su kao targeti u

simulacijama molekularnog dokinga radi ispitivanja predikcije interakcija između liganada i receptora.

Rezultati: Većina liganada nije ostvarila značajne interakcije sa ispitivanim proteinima ($\Delta G > -7$ kCal/mol), osim u slučaju Akt proteina. Za ovaj protein identifikovan je veći broj liganada sa $\Delta G < -7$ kCal/mol. Najpotentnijih 5 liganada (salvianolna kiselina, apigenin, diosmin, mangiferin i silbin) vezivali su se za aminokiselinske sekvence koje čine aktivno mesto receptora, ukazujući na mogući inhibicioni efekat.

Zaključak: *In silico* analiza ukazuje da bioaktivne komponente mediteranske dijeta poseduju potencijal za inhibiciju Akt proteina (protein kinaze B), čime se otvara perspektiva za njihovu primenu u modulaciji osteoklastne aktivnosti i razvoju novih strategija za očuvanje zdravlja kostiju.

Ključne reči: mediteranska ishrana; bioaktivna jedinjenja; osteoblasti; osteoklasti; protein kinaza B

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ORIGINAL ARTICLE

The role and significance of cardiac magnetic resonance in hypertrophic cardiomyopathy

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Summary

Introduction: Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder of cardiomyocytes that leads to myocardial thickening. The aims of this study were to diagnose HCM in patients with hypertrophic left ventricle walls, to evaluate myocardial tissue, and to assess the degree of myocardial fibrosis using cardiac magnetic resonance (CMR).

Material and Methods: The study included 51 patients diagnosed with HCM by CMR (27 males, 24 females) and was carried out in the University Clinical Centre of Serbia, Center of Radiology. All collected patient data was obtained from official medical documentation for this retrospective observational study. CMR confirmed HCM in 51 patients based on a standard imaging protocol performed on a 1.5T Siemens scanner, with the contrast agent Gadolinium. Regarding *Late Gadolinium Enhancement* (LGE) distribution, CMR enables the detection of focal fibrosis and helps to differentiate the etiology of the hypertrophic myocardium.

Results: Left ventricular outflow tract obstruction was observed in 9 (17.6%) patients. Asymmetric HCM was noted in 41 (80.4%) patients. LGE presence was detected in 39 (76.5%) patients, most notably in the septal region (62.7%). The degree of fibrosis was estimated at a median of 6% of the left ventricular mass and a median of 11 grams of fibrosis per gram of left ventricular mass.

Conclusion: CMR as a non-invasive method represents the gold standard for myocardial tissue characterization. The detection of myocardial fibrosis, a major trigger for the development of malignant arrhythmias, positions CMR as a risk stratification method in HCM patients.

Keywords: hypertrophic cardiomyopathy, cardiac magnetic resonance, late gadolinium enhancement, degree of fibrosis, arrhythmias

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease of cardiomyocytes caused by mutations in sarcomere protein genes, which encode components of cardiac contraction and it is characterized by the absence of other diseases that could lead to myocardial thickening (1–5). It represents the most common adaptive or compensatory response protecting the heart's cardiac output (CO), resulting from the influence of physiological or pathological stimuli that can lead to heart failure (6).

In adults, HCM is defined as a left ventricular (LV) wall thickness greater than 15 mm with a ratio of septal/posterior wall thickness greater than 1.3 in normotensive patients or greater than 1.5 in those with arterial hypertension. While myocardial thickening can affect any part of the ventricle, the interventricular septum is the most commonly involved part (3).

There is a wide spectrum of phenotypic expressions of HCM, for which only cardiac magnetic resonance imaging (CMR) can provide an accurate diagnosis. Hypertrophy in HCM can be asymmetric, affecting the interventricular septum, which is the most common form of the disease (60-70%). Symmetric or concentric myocardial hypertrophy can also occur approximately in about 40% pts while the apical form of HCM is the rarest (1). Regarding the degree of left ventricular outflow tract (LVOT) obstruction, HCM is classified into obstructive and non-obstructive cardiomyopathy (2).

The advantage and accuracy of CMR is attributed to the free choice of imaging planes, the wide range of imaging techniques and the absence of harmful ionizing radiation (7). CMR as a gold standard for tissue characterization of the myocardium allows a visualization of focal or diffuse changes in myocardial tissue using novel mapping techniques. These techniques are considered one of the most significant innovations of CMR, alongside the standard ability of quantifying the degree of focal fibrosis (8). Also, CMR is considered to be one of the most important non-invasive imaging modalities for risk stratification in some cardiovascular diseases (9).

The most common conditions to be considered in differential diagnosis of HCM are athletic/sport heart, hypertensive heart disease, aortic stenosis, myocardial amyloidosis, and Fabry disease. The differential diagnosis for asymmetric myocardial thickening includes usually cardiac sarcoidosis, while for the apical hypertrophy, it's a mural thrombus (1). The most challenging clinical problem is a distinction between physiological myocardial hypertrophy (athlete's heart) and hypertrophic cardiomyopathy, which has been registered as the most common cause of non-traumatic sudden cardiac death (SCD) during the exercise in young athletes (< 35 years old) (10). SCD is definitely the most dangerous complication of HCM caused by malignant arrhythmias. However, the mechanisms of its occurrence are not fully understood

due to the synergistic effect of pro-arrhythmic factors. Myocyte disturbance and fibrosis are the most common factors, as they alter the electrophysiological properties of the heart (11). The aims of this study were to diagnose HCM in patients with hypertrophic left ventricle walls, to evaluate myocardial tissue for edema or fibrosis and to assess the degree of myocardial fibrosis using CMR.

MATERIAL AND METHODOLOGY

The study was conducted at the Center of Radiology, University Clinical Center of Serbia, Department of Magnetic Resonance Imaging. All collected patient data were taken from the official medical records. The classification of this study was as a retrospective observational study. Out of a total number of 92 patients who met the echocardiographic and ECG criteria for myocardial hypertrophy, CMR revealed hypertrophic cardiomyopathy in 51 patients. Additionally, 25 patients showed CMR signs for cardiac amyloidosis (both transthyretin amyloidosis (ATTR) and light chain (AL)), 2 with athlete's heart, 8 with aortic stenosis, and 6 of them had arterial hypertension. Only patients with hypertrophic cardiomyopathy were included in further evaluation. CMR was performed during a period from June 2022 to January 2024 and revealed HCM in 51 individuals, who represented the cohort of patients for this study. This study was approved by the Ethical Committee of Faculty of Medicine University of Belgrade number 29/IX-14, in 2016 year. Patients all signed consent for CMR examination with contrast media application. Descriptive statistical methods were used for presenting data as absolute numbers and percentages. Among the descriptive statistical methods, measures of central tendency were used such as the arithmetic mean or positional median, depending on the distribution and data type, as well as measures of variability like standard deviation.

Normality of numerical variables was assessed using the Kolmogorov–Smirnov test, where $p < 0.05$ indicated a non-normal distribution. Accordingly, normally distributed variables were compared using the independent-samples t-test, while non-normally distributed variables were analyzed using the Mann–Whitney U test. Categorical variables were compared using the Chi-square test. Correlations were evaluated using Spearman's rank correlation. The Microsoft Excel 2013 program (Redmond, Washington, USA) was used for graphical and tabular data presentation.

In our study, all patients with echocardiographically confirmed myocardial hypertrophy underwent CMR, which in this context was used as the reference diagnostic method for myocardial tissue characterization and for determining the cause of hypertrophy, and has already been established in the literature as the gold standard with optimal diagnostic accuracy. Therefore, additional diagnostic procedures such as myocardial biopsy and histological verification were not performed.

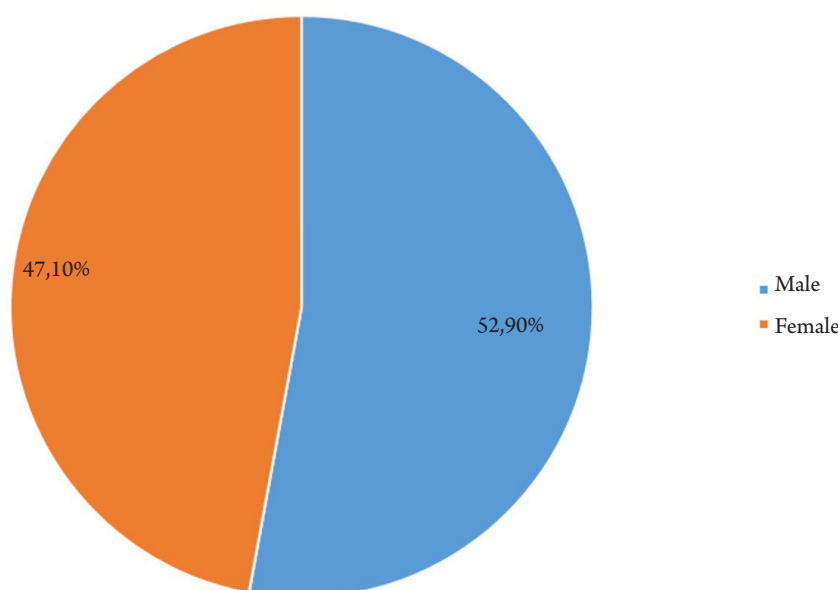


Figure 1. Sex distribution in the hypertrophic cardiomyopathy population

CMR is a multiparametric, repeatable and non-invasive high-resolution imaging technique that does not involve ionizing radiation (8,12). Based on the classical imaging protocol applied in our institution, it allows the differentiation and distinction of several diseases associated with myocardial hypertrophy. CMR was performed on 1.5 T Siemens scanner in all patients, with the administration of the contrast agent Gadolinium in order to obtain the *Late Gadolinium Enhancement* (LGE) phenomenon. The contrast agent was administered intravenously through the cubital vein at a dose of 0.2 ml/kg per body weight. The examination lasted approximately 60 minutes and it included all native images, both morphological and dynamic – cine, followed by LGE sequences acquired 10 minutes after contrast administration (8,12). Regarding the distribution of LGE phenomenon, CMR enables the detection of focal myocardial fibrosis and allows for differentiation of the etiology of the hypertrophic myocardium as we have already mentioned (8,12,13). Additionally, our study was based on a descriptive analysis of CMR findings in primary hypertrophic cardiomyopathy, a control group without myocardial hypertrophy was not included, and therefore it was not possible to calculate the sensitivity and specificity of this diagnostic procedure itself.

This study was approved by the Ethical Committee of Faculty of Medicine University of Belgrade number 29/IX-14, in 2016. Written informed consent has been obtained from the patient(s) to publish this paper.

RESULTS

In our study, there were 24 women (47.1 %) and 27 men (52.9%) (**Figure 1**).

It is typical that LV mass is higher in men than women, and this is also reflected in our study results. The

average LV mass in male was 213 g, while in female was 160 g (**Table 1**).

Female patients were significantly older (with a mean age of 61.8 ± 11.7 years) and had lower EDV, ESV, and SV in both ventricles, along with reduced LV mass (**Table 1**). No significant differences were observed in the existence of LGE ($p=0.904$) or heterogeneous myocardium ($p=0.539$) based on patient sex.

The incidence of LVOT obstruction was recorded in 9 of 51 pts (17.6%) while the majority of patients had non-obstructive cardiomyopathy (82.4%).

Of the total number of patients, asymmetric HCM was registered in 41 (80.4%) patients (**Figure 2**).

In the group of patients with asymmetric HCM, LVOT obstruction was observed in 9 (22%) patients. The apical form of HCM was identified in 10 (19.6%) patients, while concentric and symmetric HCM was not detected in our population (**Table 2**).

The LGE phenomenon was detected in the majority of patients - 39 pts (76.5%) with a predominantly mesomyocardial distribution seen in 51% of cases.

As noted in **Table 3**, LGE was detected in the septal wall in the majority of patients in nearly in 63% of cases, the anterior wall in 51% of patients, and the inferior wall in 31% of patients (**Figure 3**).

Meanwhile, the presence of LGE in the apex of left ventricle was detected in a relatively small number of patients since the apical form of HCM was not so frequent in our population. In addition to accurately determining the localization of myocardial fibrosis, the use of CMR also quantified the degree of the aforementioned myocardial fibrosis that was observed in HCM patients. In this cohort of patients, the degree of myocardial fibrosis was assessed at a median of 6% of the LV mass (values detected in the range of min 0.8, max 46.5%) and a median of

Table 1. Clinical characteristics of the patients and left ventricular parameters depending on the patient sex

	Sex		P value
	Male (n=27)	Female (n=24)	
Age (years)	54.3±12.5	61.8±11.7	0.033
Weight (kg)	86.4±13.2	72.9±13.8	0.001
BMI	26.9±3.8	25.9±5.3	0.445
BSA	2.1±0.2	1.8±0.2	<0.001
Systolic BP	120.0 (120.0-145.0)	127.0 (110.0-140.0)	0.581
Diastolic BP	80.0 (80.0-80.0)	80.0 (72.0-80.0)	0.254
LDL	3.0 (2.8-3.6)	3.0 (2.8-3.1)	0.316
HDL	1.4 (1.1-1.5)	1.5 (1.2-1.6)	0.290
Total Cholesterol	5.2 (4.7-5.6)	5.1 (4.9-5.3)	0.692
LV parameters			
Septal thickness	19.0 (17.0-22.0)	20 (13.5-23.0)	0.917
Apical thickness	8.0 (8.0-9.0)	9.0 (8.0-12.0)	0.323
EF LV	66.0 (62.0-68.0)	65.0 (60.5-67.5)	0.449
EDV	139.2±37.8	111.5±28.8	0.005
ESV	50.7±14.8	39.6±14.3	0.009
SV	89.5±26.4	71.7±17.2	0.007
Indexed EDV	69.2±16	61.2±13.9	0.064
Indexed ESV	24.2±7.2	21.7±7.2	0.221
Indexed SV	44.8±10.5	39.4±8.4	0.051
CI	2.8±0.6	2.5±0.6	0.076
LV mass	213.5±52.7	160.5±47.3	0.001
Left Atrium Area	19±4.5	19.4±4.5	0.736
RV parameters			
EF RV	66.2±6	66.2±4.7	0.988
EDV	129.4±23.7	104±19.7	<0.001
ESV	44.3±12.4	35.2±8	0.003
SV	85.6±16.3	69.1±14.8	<0.001
Indexed EDV	63.0 (55.0-73.0)	60.5 (52.0-64.0)	0.245
Indexed ESV	21.0 (15.0-28.0)	20.5 (17.0-22.0)	0.324
Indexed SV	41.9±8.4	39.5±12.8	0.243
CI	2.7 (2.3-3.0)	2.5 (2.0-2.9)	0.254
Right Atrium Area	16.0(15.0-18.0)	15.0 (14.0-16.0)	0.044

BMI – Body Mass Index; BSA - Body Surface Area; BP - Blood pressure; LDL - Low-density lipoprotein; HDL - High-density lipoprotein; LV - Left ventricle; EF - Ejection fraction; EDV - End-diastolic volume; ESV - End-systolic volume; SV - Stroke volume; CI - Cardiac index; RV - Right ventricle. Data are presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for non-normally distributed variables. The independent-samples t-test was performed for normally distributed variables and the Mann–Whitney U test for non-normal variables. Significant p values (less than 0.05) are marked in bold font.

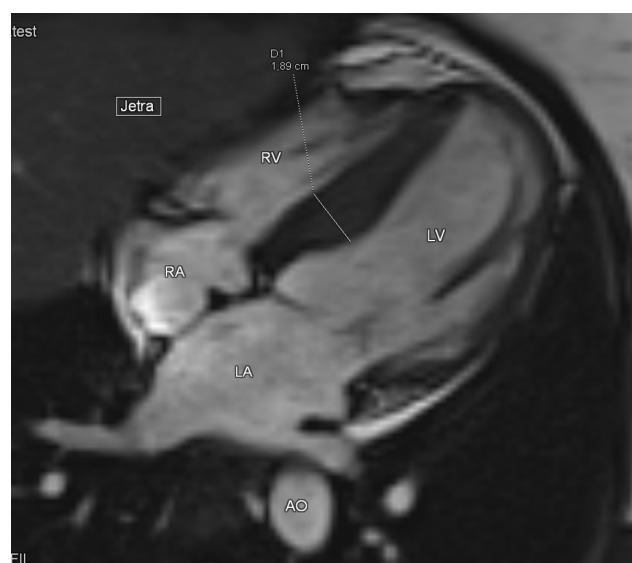

Figure 2. Cardiac magnetic resonance (CMR) cine sequences 4 chamber view with no contrast media presenting asymmetric septal hypertrophy of left ventricle (septal wall 19 mm). No patient-identifying features are visible

Table 2. Morphological forms of HCM.

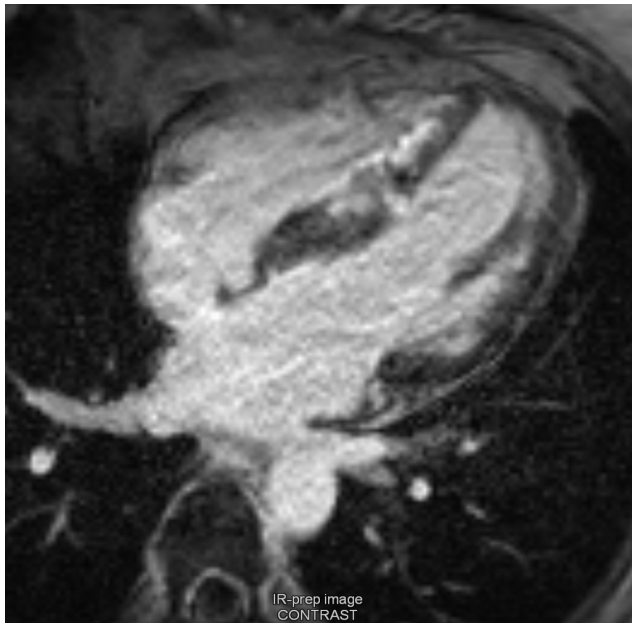
	Frequency	Percentage (%)
Asymmetric HCM	41	80.4
Apical HCM	10	19.6
Concentric HCM	0	0
Total	51	100.0

HCM – Hypertrophic Cardiomyopathy

Table 3. LGE phenomenon distribution in left ventricle

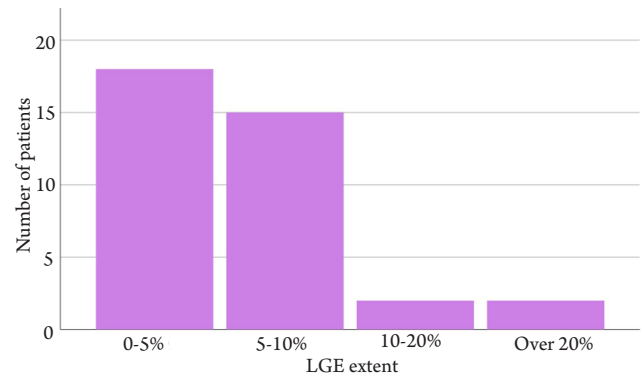
Presence of LGE phenomenon localization	Number of pts	Percentage (%)
Septum	32	62.7
Lateral wall	13	25.5
Anterior wall	26	51.0
Insertion point	12	23.5
Apical	9	17.6
Inferior wall	16	31.4
Posterior wall	7	13.7

LGE - Late Gadolinium Enhancement

**Figure 3.** Cardiac magnetic resonance (CMR) 4 chamber view long axis after contrast media application (Gadolinium) showing mesomyocardial LGE phenomenon in septal wall. No patient-identifying features are visible

11 g of fibrosis per gram of LV mass (values detected in the range of 1.0–135.8 g). The majority of patients exhibited LGE values of up to 5% (**Figure 4**), with the minimum recorded value at 0.8% and the maximum at 46.5%. Only 3 patients (6%) from the cohort had a degree of focal fibrosis that presented as a LGE phenomenon of greater than 15%.

The overall severity of LGE showed a moderate, significant positive correlation with septal thickness ($r_s=0.458$, $p=0.004$, **Figure 5**), whereas no correlation was observed between LGE extent and apical thickness ($r_s=0.094$, $p=0.581$, **Figure 6**). Moreover, the extent of LGE presented with a moderate, significant negative correlation with LV EF ($r_s=-0.512$, $p=0.001$, **Figure 7**).

**Figure 4.** LGE extent expressed as percentage of myocardial mass in patients with HCM

In addition to focal fibrosis, which was most frequently registered in our patients as a LGE phenomenon, there were also patients with diffusely heterogeneous myocardium after administration of Gadolinium contrast media, and that was seen in 10 patients, representing about 20% of the cohort.

DISCUSSION

In this retrospective study analyzing patients with HCM, there was a slightly higher number of male patients 27 (53%). In the study of Abraham and colleagues, which included a systematic review of data from 37700 patients, it was shown that there was no statistically significant difference in the incidence of HCM between men and women (8, 14). However, in the study of Angkawipa et al, it was demonstrated that HCM was more prevalent among men (15). It is known that the LV mass is greater in men than in women, which is also seen in our population.

In our research, the most common form of HCM was asymmetric HCM observed in 80% of participants. The study by Wenn Xu and colleagues also showed that asymmetric HCM had been the most frequent form of HCM (60–70%) (12). In our population with asymmetric HCM LVOT obstruction was recorded in 22% of patients.

The data from our study differ from those of the study by Song et al, that reported on a significantly larger number of patients with HCM (758 subjects) and showed that obstruction of LVOT occurred in the majority of patients, present in almost two-thirds of the participants (16).

Regarding the presence and assessment of the degree of myocardial fibrosis in HCM patients, we observed the presence of pathological accumulation of contrast media in a form of LGE phenomenon (17). However, as previously noted, the absence of LGE phenomenon does not imply the absence of fibrosis, as LGE only indicates the presence of focal myocardial fibrosis (18). In our study, LGE was detected in a vast majority of patients, in about 77% of cases.

In the study of Aquaro et al, myocardial fibrosis in the form of the LGE phenomenon was also found in over 60%

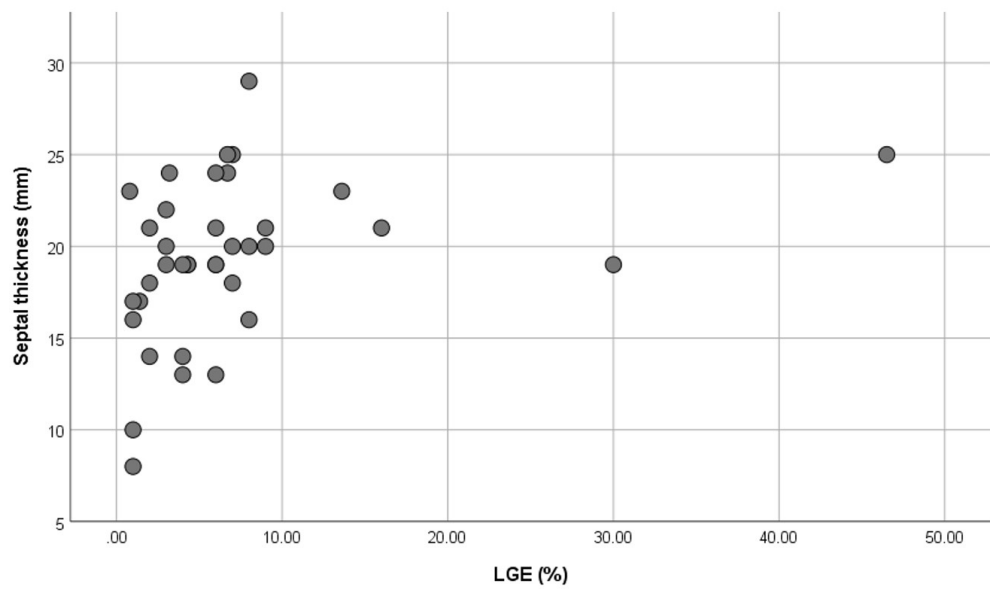


Figure 5. Correlation of left ventricular late gadolinium enhancement (LGE) extent with septal thickness.

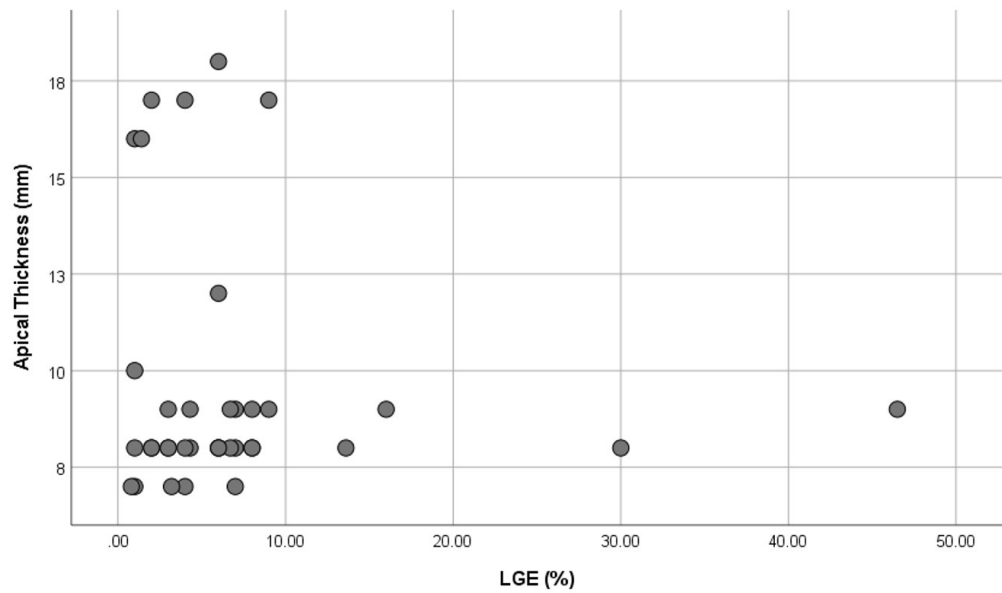


Figure 6. Correlation of left ventricular late gadolinium enhancement (LGE) extent with apical thickness.

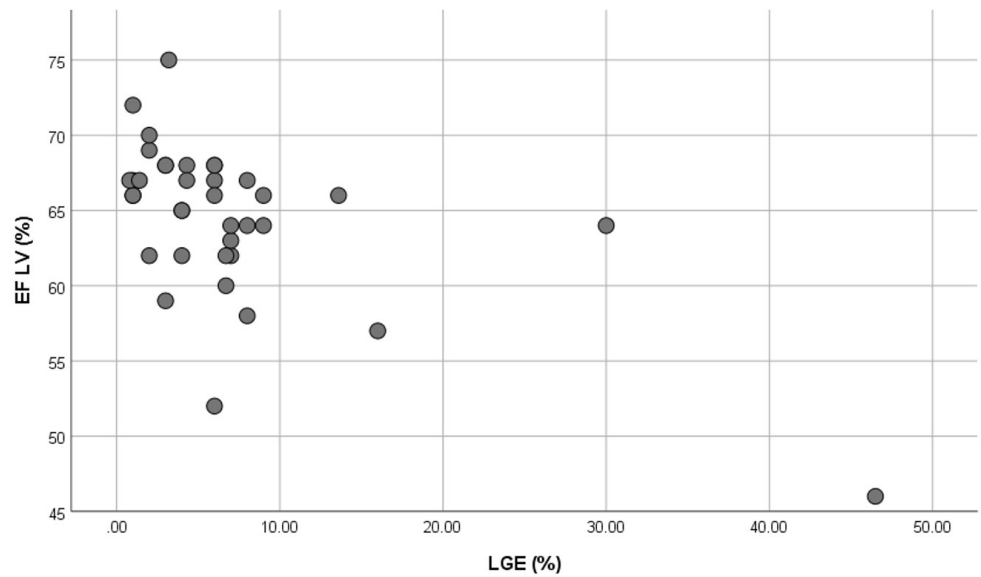


Figure 7. Correlation of left ventricular late gadolinium enhancement (LGE) extent left ventricle (LV) ejection fraction (EF).

of cases with HCM, and it was considered as a marker of replacement focal fibrosis (19). In the study of Maloch et al, which included 50 patients with HCM, myocardial fibrosis expressed through the LGE phenomenon was noted to occur in 37 (74%) patients, which aligns with the results of our sample (20). However, in the study of Prinz et al, which included 87 patients, about 90% of participants had LGE phenomenon (21).

In our population, the most common localization of LGE was in the interventricular septal wall (63%), then in the anterior wall (51%), and the inferior wall (31%). In the study by Liu et al, was shown also that myocardial fibrosis was predominantly distributed in the interventricular septum and in the anterior wall (22), which is consistent with our findings. Studies by Barbosa et al., Rodrigues, Machii, Sultan, as well as Chan and co-authors, have demonstrated that the mid-septal wall was the most frequent localization for detecting the LGE phenomenon (23–26).

In our study, a heterogeneous myocardium, meaning myocardium with diffuse interstitial fibrosis and with increased values of native T1 mapping, was observed in 10 patients (20%). In the study by Maloch et al., heterogeneous myocardium was present in 28% of the 54 analyzed cases (20), which is in accordance with our results.

The presence of focal myocardial fibrosis presented as LGE phenomenon seen on CMR is considered one of the important predictive factors associated with an increased risk of adverse outcomes and SCD (27–31). Various studies have shown that in patients with confirmed LGE phenomenon detected on CMR, there is a higher incidence of malignant arrhythmias, SCD, and all-cause mortality. The study by Maloch et al. showed a positive correlation between the degree of myocardial fibrosis and frequency of ventricular tachycardia (20). Extensive LGE seen on CMR could be used as a risk stratification factor for traditional SCD in patients with HCM as well as for better understanding their overall risk of ventricular arrhythmias (32, 33). It was previously considered that LV wall thickness brings to a higher incidence of ICD therapy than other traditional SCD risk factors (32). But new studies have shown that the presence of massive focal myocardial fibrosis, however, identifies a subgroup of patients with no large myocardial wall hypertrophy but with an equally high incidence of receiving antiarrhythmic device therapy (34).

In addition, the study of Rader and co-authors found that the presence of extensive LGE phenomenon with more than 15% fibrosis of myocardial mass was associated with a higher risk of SCD compared to patients with a lower percentage of LGE in HCM patients (31, 35).

In our study, the degree of myocardial fibrosis was estimated at a median of 6% of LV mass, and only 3 patients (6%) out of 51 had an extensive and high degree of myocardial fibrosis in the form of LGE phenomenon exceeding 15% of LV mass. All those three patients, after undergoing consultant review, received Implantable Cardioverter Defibrillators (ICD) in order to reduce the risk of SCD.

According to study by Sebastian and Alexandra, the incidence of atrial fibrillation (AF) in patients with HCM was 22% in both sexes. Also, they demonstrated that women have a moderately increased risk for AF, as well as for myocardial fibrosis (36, 37).

CONCLUSION

This study represents an important contribution for understanding the role of CMR in diagnosis of HCM. CMR is a non-invasive diagnostic method that is considered as a gold standard for myocardial tissue characterization. It plays a crucial role not only in the diagnosis of HCM, but also in the differential diagnosis of myocardial hypertrophy, allowing for accurate distinction between pathological and physiological hypertrophy, as well as infiltrative or pressure-overload conditions that can mimic HCM. CMR provides high-quality visualization of cardiac morphology as well as functional assessment, enabling further treatment planning. It is important to emphasize that CMR offers numerous benefits, not only in terms of tissue characterization without the use of ionizing radiation, but also in the non-invasive measurement of volumetric parameters of the heart chambers. The detection of myocardial fibrosis, as a main trigger for the development of malignant cardiac arrhythmias, places CMR not only as a diagnostic tool, but also as a risk stratification method to evaluate the risk SCD in these patients.

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ULOGA I ZNAČAJ MAGNETNE REZONANCE SRCA KOD HIPERTROFIČNE KARDIOMIOPATIJE

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Sažetak

Uvod: Hipertrofična kardiomiopatija (engl. *hypertrophic cardiomyopathy* – HCM) je autozomno dominantna bolest kardiomiocita koja dovodi do njihovog zadebljanja i hipertrofije. Ciljevi ovog rada su bili da se dijagnostikuje hipertrofična kardiomiopatija pomoću magnetne rezonance (MR) srca kod pacijenata sa hipertrofijom miokarda leve komore, okarakterise tkivo miokarda i odredi stepen fibroze ovom dijagnostičkom metodom.

Metode: Studija je obuhvatila 51 pacijenta sa HCM dijagnostikovanom na MR srca (muškarci 27; žene 24) i sprovedena je na Univerzitetskom kliničkom centru Srbije, Centru za radiologiju. Svi prikupljeni podaci o pacijentima su uzeti iz zvanične medicinske dokumentacije, a tip studije je retrospektivna opservaciona studija. MR srca je kod 51 pacijenta pokazao postojanje HCM na osnovu klasičnog protokola snimanja rađenog na 1.5T Siemens aparatu uz davanje kontrastnog sredstva Gadolinijuma. U odnosu na distribuciju kontrasta u vidu *Late Gadolinium*

um Enhancement (LGE) MR srca omogućava otkrivanje fokalne fibroze, a samim tim i razlikovanje etiologije hipertrofičnog miokarda.

Rezultati: Opstrukcija izlaznog trakta leve komore viđena je kod 9 (17.6%) pacijenata. Asimetrična HCM je registrovana kod 41 (80.4%) bolesnika. Prisustvo LGE detektovano je kod 39 (76,5%) pacijenata, najviše u predelu septuma (62.7%). Stepenn fibroze procenjen je na medianu od 6% mase leve komore i medianu od 11 g fibroze po gramu mase leve komore.

Zaključak: MR srca kao neinvazivna metoda predstavlja zlatni standard u tkivnoj karakterizaciji srčanog mišića. Detekcija fibroze miokarda i njeno kvantifikovanje, kao glavnog okidača za razvoj malignih srčanih aritmija, svrstava MR srca, ne samo u dijagnostički, već i u metod stratifikacije rizika od naprasne srčane smrti kod pacijenata sa hipertrofičnom kardiomiopatijom.

Ključne reči: hipertrofična kardiomiopatija, magnetna rezonanca srca, kasno nakupljanje gadolinijuma, stepen fibroze, aritmije

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Medicinska istraživanja 2026

ORIGINAL ARTICLE

Assessment of left main coronary artery luminal diameter using quantitative coronary analysis and optical coherence tomography

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Summary

Introduction: Accurate assessment of left main (LM) coronary lumen dimensions is essential during percutaneous coronary intervention (PCI). Quantitative coronary analysis (QCA) is routinely used, yet its agreement with high-resolution intravascular imaging modalities such as optical coherence tomography (OCT) in the LM segment remains incompletely defined.

Methods: Thirty patients who underwent LM PCI were evaluated using follow-up angiography and OCT. Eight matched lumen parameters were assessed using 2D QCA, 3D QCA, and OCT. Differences between modalities were analyzed using repeated-measures ANOVA for normally distributed variables and Friedman's test for minimal lumen area (MLA). The correlations were assessed descriptively, and agreement was evaluated using Bland-Altman analysis.

Results: Significant modality-dependent differences were observed across most lumen parameters. QCA demonstrated strong associations with OCT for reference- and mean-based lumen metrics, whereas correlations were weaker for extreme (minimal and maximal) dimensions. Bland-Altman analysis revealed minor mean differences between QCA and OCT but wide limits of agreement, indicating substantial inter-individual variability despite consistency at the group level.

Conclusion: QCA provides reasonably consistent estimates of reference and mean lumen dimensions in the LM segment but shows limited association with OCT for extreme measurements. Despite strong associations, individual variability remains considerable, supporting OCT as the preferred modality for precise lumen assessment, stent sizing, and procedural optimization in LM PCI.

Keywords: optical coherent tomography, quantitative coronary angiography, left main coronary artery, correlation analysis, lumen sizing

INTRODUCTION

Precise assessment of coronary lumen dimensions is fundamental for planning and evaluating percutaneous coronary intervention (PCI), especially in anatomically large and clinically critical segments such as the left main coronary artery (LMCA) (1, 2). Quantitative coronary analysis (QCA) remains the most widely used technique for quantifying angiographic lumen size in routine practice (3). Modern QCA software provides both two-dimensional (2D) measurements, such as reference and minimal diameter, as well as three-dimensional (3D) reconstructions, enabling the calculation of lumen areas, including reference, minimal, and mean cross-sectional areas (3). On the other hand, the LMCA is a short vessel segment that frequently exhibits plaque and lumen asymmetry, complex bifurcation geometry, and significant tapering (4). These anatomical features introduce variability in 2D and 3D QCA measurements and restrict the accuracy of angiographic assessment (3). Consequently, QCA may underestimate accurate lumen dimensions, particularly in large-caliber or non-cylindrical regions, and may be influenced by projection angle, vessel eccentricity, and foreshortening (5). Optical coherence tomography (OCT), together with intravascular ultrasound (IVUS), is established as the reference imaging modality for intracoronary lumen evaluation due to its high spatial resolution and ability to provide accurate cross-sectional measurements (1, 2). OCT allows precise quantification of diameter, minimal and mean lumen areas, and geometric characteristics of bifurcation segments, making it the ideal modality for comparing QCA measurements (1, 2). Although the correlation between angiographic and intravascular imaging metrics has been demonstrated in other coronary segments, systematic evaluation of these relationships specifically within the LMCA remains limited. Therefore, a comprehensive comparison of QCA-derived lumen variables with OCT findings would enable identification of which angiographic parameters most closely reflect OCT measurements, where systematic discrepancies occur, and how QCA can be used

more reliably in settings where intravascular imaging is unavailable. Understanding inter-modality differences and levels of agreement is clinically relevant for procedural planning and stent sizing in left main PCI.

This study aimed to compare quantitative measurements obtained with 2D QCA, 3D QCA, and OCT, and to assess their agreement and potential clinical interchangeability in assessing left main coronary artery lesions.

METHOD

Sample

This study included 30 patients who underwent invasive follow-up with OCT control after primary PCI of the LMCA guided only by angiography and remained free of adverse events during long-term follow-up. Given the exploratory nature of the study, no formal sample size calculation was performed. The sample size was determined by the number of patients with complete paired angiographic and OCT datasets available for analysis.

Ethical approval and informed consent

This study was approved by the University Clinical Centre Ethical Committee (1500/34/2025; September 29, 2025), by the Council of Scientific Field of Medical Sciences, of the Faculty of Medicine, University of Belgrade (61206-4744/2-21, September 30, 2021), and the Research Board of the Department of cardiology of the University Clinical Center of Serbia (review No: 1883/21, August 24, 2021). All participants provided written informed consent before enrollment and before undergoing any study-related procedures.

Lumenometric analysis

A unified set of eight measurements was calculated using both QCA and OCT, enabling direct one-to-one comparison between modalities. These included: minimal lumen

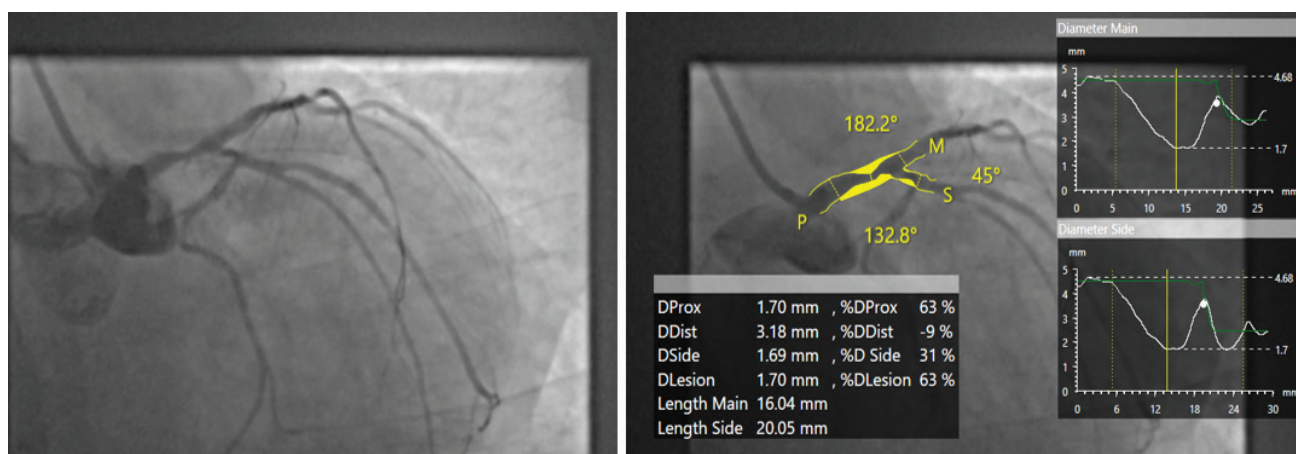


Figure 1. Left panel – caudal projection, still frame selected during coronarography; Right panel – 2D QCA analysis from the chosen projection and frame.

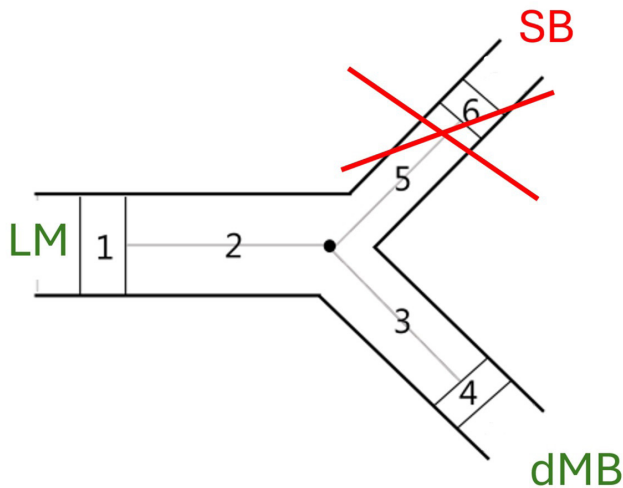


Figure 2. QCA segment analysis: 1 – reference segment of LM, 2 – LM segment, 3 – dMB segment, 4 – reference of dMB segment, 5 and 6 SB segments.

Abbreviations: dMB – distal main branch; QCA – quantitative coronary analysis; LM – left main; SB – side branch.

diameter (MLD), maximal lumen diameter (MaxD), mean lumen diameter (MeanD), reference diameter (RD), minimal lumen area (MLA), reference lumen area (RefA), maximal lumen area (MaxA), and mean lumen area (MeanA).

QCA analysis

CAAS Workstation 7.3 with the bifurcation module was used for QCA measurements. To guarantee complete contrast filling and no vessel overlap, angiographic projections with the best LMCA visualization were chosen for 2D QCA. The 6F or 7F catheter filled with contrast was used for calibration. After tracing the vessel's centerline, automated edge detection was used to define the LMCA contours. When necessary, manual contour correction was used, but it was kept to less than 15% of the segment length (**Figure 1**).

Reference diameter was defined from a 5-mm normal reference segment proximal and distal to the lesion, or the ostium when applicable. Segmental analysis was performed using the six-segment model integrated into the software, allowing extraction of all lumenometric parameters. Since OCT analysis was available only for the LM and distal main branch (dMB) segments (segments 5 and 6), corresponding to the side-branch (SB) region, these segments were excluded from further analysis (**Figure 2**).

The identical analysis was repeated for 3D QCA in a second projection that was more than 30° apart. By combining the two perspectives, the software produced a 3D reconstruction with spatially corrected measurements (**Figure 3**). All lumenometric variables were extracted from the 3D model of the LMCA bifurcation (**Figure 4**).

OCT analysis

OCT pullbacks were recorded with standard contrast-flush acquisition. Quantitative lumenometric analysis was performed using CAAS Intravascular at 0.2-mm cross-section intervals across the LMCA bifurcation. All frames were reviewed for image quality, and sections with inadequate visualization or flushing artifacts were excluded.

To match the QCA results export in two segments, OCT analysis was also performed using a two-segment LMCA model: LM and distal main branch (dMB) (**Figure 5**).

For each segment, the same eight lumenometric parameters were extracted: MLD, MaxD, RD, MeanD, MLA, RefA, MaxA, and MeanA.

Statistical analysis

Normality of continuous variables was assessed using visual inspection of histograms and Q–Q plots, as well

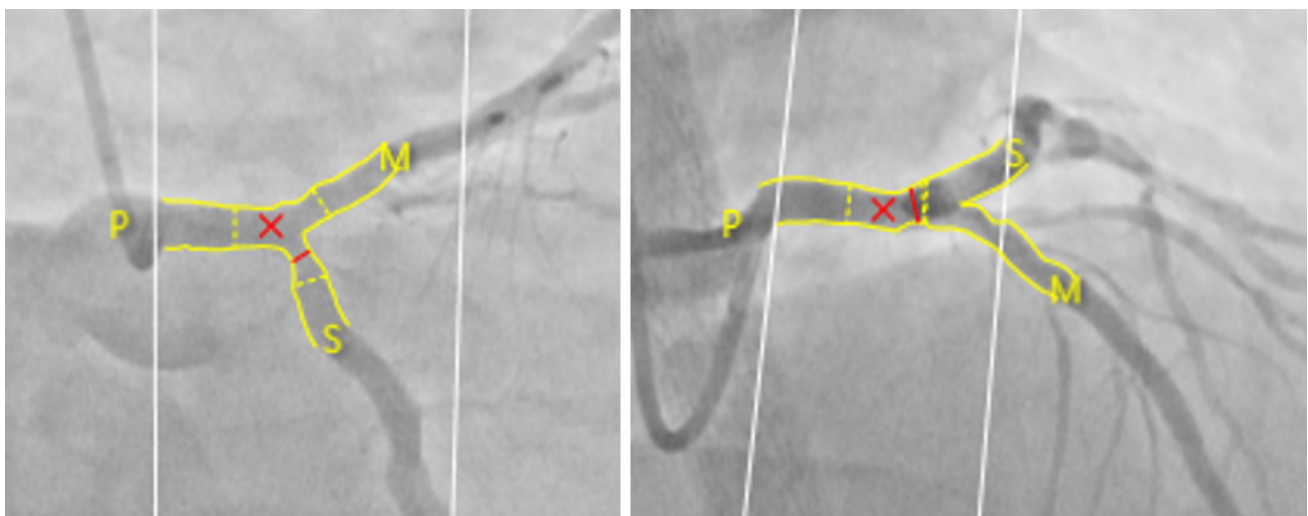


Figure 3. 3D QCA analysis. Left panel – caudal projection analysis; Right panel – cranial projection analysis.

Abbreviations: 3D QCA – three-dimensional quantitative coronary analysis

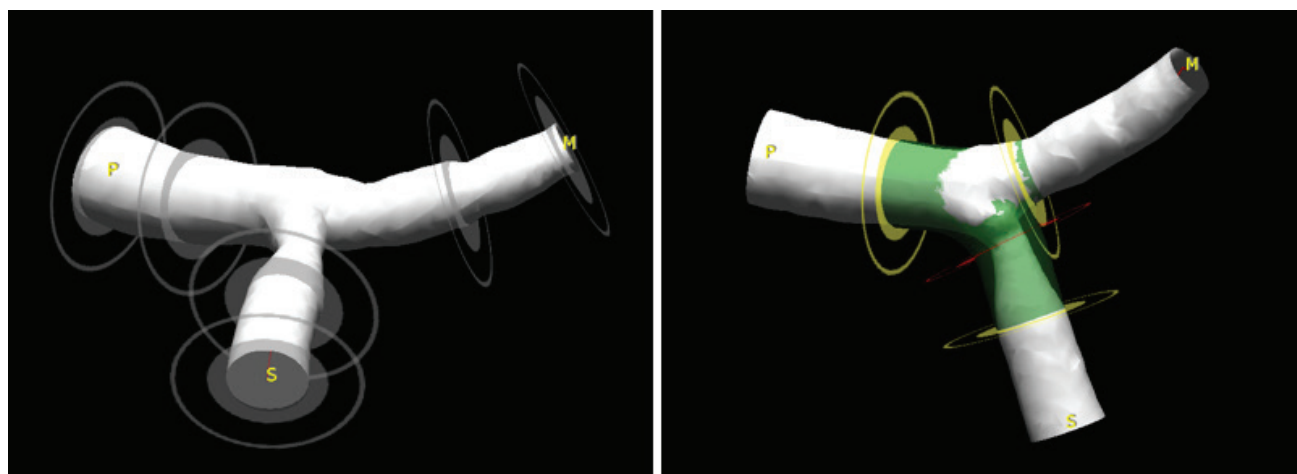


Figure 4. 3D model of LMCA generated during 3D QCA analysis.

Abbreviations: LMCA – left main coronary artery; 3D QCA – three-dimensional quantitative coronary analysis.

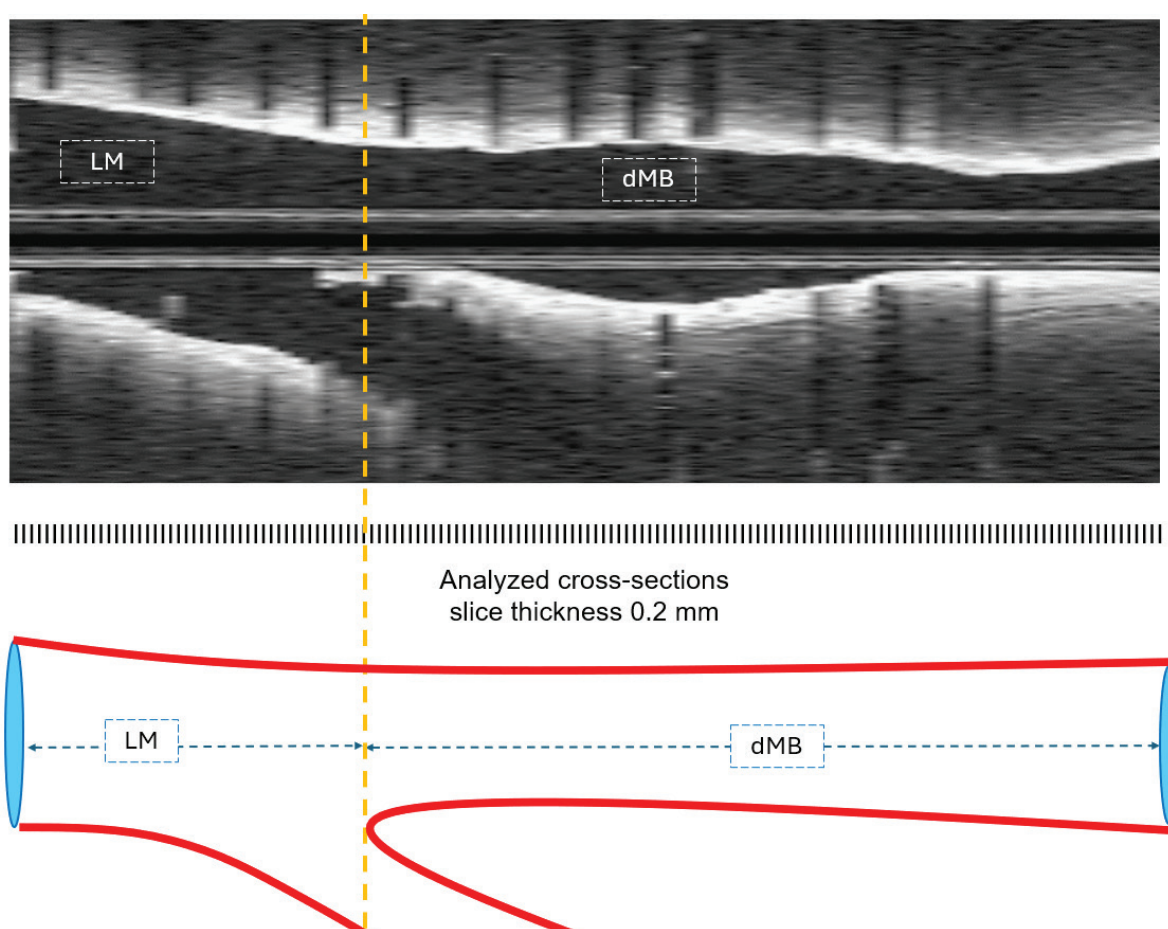


Figure 5. OCT analysis adapted for bifurcation anatomy.

Abbreviations: dMB – distal main branch; LM – left main.

as the Shapiro–Wilk test. Continuous variables are presented as mean $\pm$ standard deviation, whereas MLA is presented as median with interquartile range due to non-normal distribution. Differences between measurement modalities were assessed using a repeated-measures ANOVA with Greenhouse–Geisser correction for normally distributed variables and Friedman’s test for MLA due to non-normality. Post-hoc pairwise comparisons were adjusted for multiple testing using Bonferroni

correction. Correlation analyses (Pearson for normally distributed variables and Spearman for MLA) were used for descriptive purposes only to characterize the strength of correlation between measurements obtained by different imaging modalities and do not imply agreement. Agreement between modalities was primarily assessed using Bland–Altman analysis, including calculation of mean bias and 95% limits of agreement. Statistical significance was defined as $p < 0.05$. Statistical analyses were

performed using IBM SPSS Statistics (version 26.0; IBM Corp., Armonk, NY, USA) and Python (version 3.1), using standard scientific libraries for statistical analysis and data visualization.

RESULTS

The study population consisted of 30 patients undergoing quantitative coronary analysis and optical coherence tomography of the left main coronary artery. The cohort included predominantly male patients (74%), with a mean age of 66.1 ± 13.7 years. Two-dimensional QCA was performed in all 30 patients, whereas three-dimensional QCA was performed in 29 cases due to insufficient numbers of suitable angiographic projections. A total of 29 OCT pullbacks were included in the final analysis, with one recording excluded due to suboptimal image quality. Paired 2D QCA, 3D QCA, and OCT measurements enabled direct within-patient comparisons across imaging modalities for the available datasets. *Lumenometric analysis*

The lumenometric assessment revealed significant systematic differences between 2D QCA, 3DQCA, and OCT across all anatomical segments (**Table 1**).

Left main sub-segment

In the LM subsegment, OCT identified significantly lower MLD values compared to QCA modalities, while simultaneously recording the highest MaxD. This divergence highlights OCT's superior sensitivity for detecting vessel eccentricity in the proximal segment, where angiographic projections often underestimate the major axis of elliptical lumens.

Distal main branch (dMB) sub-segment

In the dMB subsegment, 3DQCA consistently yielded the most conservative estimates for luminal areas, suggesting a systematic bias toward smaller vessel dimensions in 3D reconstructions.

Combined LM + dMB analysis

Notably, the combined analysis (LM + dMB) yielded results comparable to those of 2D QCA and OCT for area estimation, with no statistically significant differences observed for Reference Area and MLA. This suggests that, despite differences in linear-diameter measurements, 2D QCA remains a robust surrogate for cross-sectional area estimation across the overall bifurcation. In contrast, 3D QCA significantly underestimated these parameters compared to both 2D QCA and OCT, reinforcing its role as the most restrictive modality in bifurcated lesion assessment.

Correlation analysis

Overall, correlations with OCT were strongest in the LM segment and weaker in the dMB, with consistently higher correlations for 3D QCA compared with 2D QCA, particularly in distal measurements. Across segments, the strongest associations with OCT were observed for reference-based and global lumen indices, whereas correlations for minimal and focal diameter parameters were more variable.

In the LM segment, both QCA techniques showed strong correlations with OCT, particularly for mean diameter and area-based parameters, including MLA and meanA. In contrast, correlations in the dMB were generally attenuated, with several nonsignificant associations, especially for reference diameter and reference area, reflecting increased variability in distal segments. When LM and dMB were analyzed together, correlations were highest and most consistent, confirming a stable association between QCA- and OCT-derived measurements across the whole left main bifurcation anatomy (**Table 2**).

Bland-Altman analysis was performed to assess agreement between 2D and 3D QCA and OCT for the two most clinically relevant luminal parameters used for PCI guidance and stent sizing, namely RD and RefA. Scatter plots were used to illustrate the linear association between measurements. Scatter analysis demonstrated significant positive correlations between QCA and OCT for both RD and RefA (**Figure 6**). For RD, both 2D and 3D QCA showed strong linear associations with OCT, with slightly higher correlation coefficients observed for 3D QCA and a visually tighter distribution of data points. For RefA, similarly strong correlations were observed for both QCA techniques across the range of values assessed. Despite these significant correlations, scatter plots suggested a tendency toward underestimation by both QCA methods at larger lumen sizes. Overall, these findings indicate a stable linear relationship between QCA- and OCT-derived measurements, while highlighting potential systematic differences at higher values. These findings reflect a linear association rather than agreement.

Bland-Altman analysis for 2D QCA demonstrated a slight mean bias relative to OCT for both RD and RefA (**Figure 7**). However, the limits of agreement were wide, indicating substantial variability at the individual level. For RD, the observed dispersion suggests that deviations of a magnitude potentially relevant to procedural decision-making may occur, whereas for RefA, variability increased with larger lumen dimensions. For 3D QCA, the mean bias remained similarly low, and the limits of agreement were consistently narrower than in 2D QCA, indicating improved geometric agreement with OCT. Nevertheless, clinically relevant dispersion persisted for both RD and RefA, particularly at higher values, suggesting that individual measurements may still deviate mean-

Table 1. Lumenometric analysis, comparison between 2D QCA, 3D QCA, and OCT measurements.

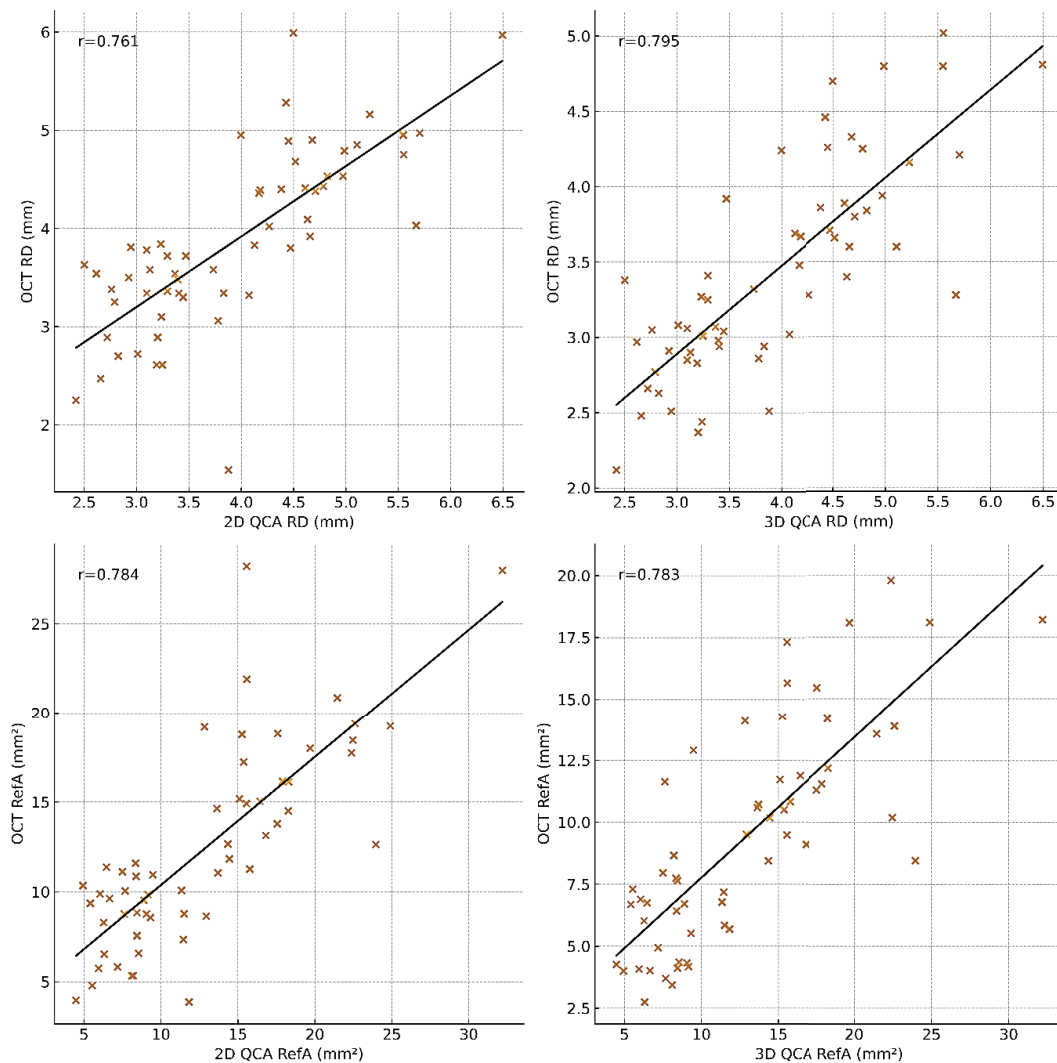
LM segment	2D QCA	3D QCA	OCT	p (all)	p 2D–3D	p 2D–OCT	p 3D–OCT
MLD (mm, mean±SD)	3.6±0.8	3.5±0.7	2.7±0.7	<0.001	0.121	<0.001	<0.001
MaxD (mm, mean±SD)	4.7±0.7	4.3±0.5	5.6±0.9	<0.001	<0.001	<0.001	<0.001
MeanD (mm, mean±SD)	4.1±0.6	3.8±0.6	3.6±0.7	<0.001	<0.001	<0.001	0.108
RD (mm, mean±SD)	4.6±0.7	3.9±0.6	4.7±0.7	<0.001	<0.001	0.176	<0.001
MLA (mm ² , median [IQR])	10.8 [8.4–13.5]	9.9 [7.9–11.7]	10.0 [7.8–14.2]	0.084	0.086	0.696	0.123
RefA (mm ² , mean±SD)	16.8±5.4	12.5±3.5	17.7±4.9	<0.001	<0.001	0.18	<0.001
MaxA (mm ² , mean±SD)	18.1±5.3	14.7±3.6	17.9±4.9	<0.001	<0.001	0.721	<0.001
MeanA (mm ² , mean±SD)	13.4±3.5	12.1±3.4	13.7±4.1	0.010	0.017	0.476	0.010
dMB segment	2D QCA	3D QCA	OCT	p (all)	p 2D–3D	p 2D–OCT	p 3D–OCT
MLD (mm, mean±SD)	2.9±0.5	2.5±0.4	2.4±0.5	<0.001	<0.001	<0.001	0.234
MaxD (mm, mean±SD)	4.3±0.5	3.9±0.5	3.9±0.5	0.002	0.001	0.004	0.862
MeanD (mm, mean±SD)	3.3±0.4	3.0±0.4	2.9±0.4	<0.001	<0.001	<0.001	0.020
RD (mm, mean±SD)	3.2±0.6	2.9±0.4	3.2±0.4	0.014	0.002	0.808	0.009
MLA (mm ² , median [IQR])	6.5 [4.6–7.4]	4.9 [4.1–6.1]	6.4 [5.2–7.4]	<0.001	<0.001	0.849	0.005
RefA (mm ² , mean±SD)	8.6±2.5	6.2±2.4	8.1±2.1	<0.001	<0.001	0.59	<0.001
MaxA (mm ² , mean±SD)	14.6±3.7	11.8±3.8	9.7±2.1	<0.001	0.005	<0.001	0.006
MeanA (mm ² , mean±SD)	8.8±2.2	6.7±1.5	7.9±1.7	<0.001	<0.001	0.001	<0.001
Combined LM + dMB	2D QCA	3D QCA	OCT	p (all)	p 2D–3D	p 2D–OCT	p 3D–OCT
MLD (mm, mean±SD)	3.2±0.8	3.0±0.7	2.6±0.6	<0.001	<0.001	<0.001	<0.001
MaxD (mm, mean±SD)	4.5±0.7	4.1±0.6	4.8±1.1	<0.001	<0.001	0.017	<0.001
MeanD (mm, mean±SD)	3.7±0.6	3.4±0.6	3.2±0.7	<0.001	<0.001	<0.001	0.009
RD (mm, mean±SD)	3.9±0.9	3.4±0.7	4.0±1.0	<0.001	<0.001	0.446	<0.001
MLA (mm ² , median [IQR])	7.6 [5.7–10.8]	6.4 [4.8–9.7]	7.5 [6.1–10.4]	<0.001	0.001	0.633	0.003
RefA (mm ² , mean±SD)	12.7±5.9	9.3±4.4	12.9±6.1	<0.001	<0.001	0.405	<0.001
MaxA (mm ² , mean±SD)	16.4±4.9	13.3±3.9	13.7±5.6	<0.001	<0.001	<0.001	0.597
MeanA (mm ² , mean±SD)	11.1±3.7	9.4±3.8	10.7±4.3	<0.001	<0.001	0.189	<0.001

Abbreviations: 2D QCA – two-dimensional quantitative coronary analysis; 3D QCA – three-dimensional quantitative coronary analysis; dMB – distal main branch; IQR – inter-quartile range; MaxA - maximal lumen area; MaxD - maximal lumen diameter; MeanA - mean lumen area; MeanD - mean lumen diameter; MLA - minimal lumen area; MLD - minimal lumen diameter; LM – left main; OCT – optical coherence analysis; RD - reference diameter; RefA - reference lumen area; SD – Standard deviation.

Table 2. Correlation analysis between 2D QCA, 3D QCA, and OCT measurements.

LM	MLD r (p)	MaxD r (p)	MeanD r (p)	RD r (p)	MLA ρ (p)	RefA r (p)	MaxA r (p)	MeanA r (p)
2D QCA vs. OCT	0.509 (p<0.001)	0.689 (p<0.001)	0.779 (p<0.001)	0.607 (p<0.001)	0.841 (p<0.001)	0.593 (p<0.001)	0.747 (p<0.001)	0.794 (p<0.001)
3D QCA vs. OCT	0.661 (p<0.001)	0.472 (p=0.013)	0.711 (p<0.001)	0.597 (p<0.001)	0.725 (p<0.001)	0.569 (p=0.001)	0.566 (p=0.002)	0.725 (p<0.001)
dMB	MLD r (p)	MaxD r (p)	MeanD r (p)	RD r (p)	MLA ρ (p)	RefA r (p)	MaxA r (p)	MeanA r (p)
2D QCA vs. OCT	0.287 (p<0.001)	0.476 (p<0.001)	0.572 (p<0.001)	0.110 (p<0.001)	0.465 (p<0.001)	0.169 (p<0.001)	0.582 (p<0.001)	0.667 (p<0.001)
3D QCA vs. OCT	0.493 (p<0.001)	0.486 (p<0.001)	0.715 (p<0.001)	0.288 (p<0.001)	0.484 (p<0.001)	0.279 (p<0.001)	0.202 (p<0.001)	0.538 (p<0.001)
LM + dMB	MLD r (p)	MaxD r (p)	MeanD r (p)	RD r (p)	MLA ρ (p)	RefA r (p)	MaxA r (p)	MeanA r (p)
2D QCA vs. OCT	0.549 (p<0.001)	0.633 (p<0.001)	0.809 (p<0.001)	0.761 (p<0.001)	0.751 (p<0.001)	0.784 (p<0.001)	0.690 (p<0.001)	0.856 (p<0.001)
3D QCA vs. OCT	0.613 (p<0.001)	0.521 (p=0.001)	0.605 (p<0.001)	0.795 (p<0.001)	0.785 (p<0.001)	0.783 (p<0.001)	0.511 (p<0.001)	0.848 (p<0.001)

Abbreviations: 2D QCA – three-dimensional quantitative coronary analysis; 3D QCA – three-dimensional quantitative coronary analysis; dMB – distal main branch; MaxA - maximal lumen area; MaxD - maximal lumen diameter; MeanA - mean lumen area; MeanD - mean lumen diameter; MLA - minimal lumen area; MLD - minimal lumen diameter; LM – left main; OCT – optical coherence analysis; RD - reference diameter; RefA - reference lumen area; r – Pearson correlation coefficient; ρ – Spearman rank correlation coefficient.

**Figure 6.** Upper left panel – scatter plot 2D QCA-OCT for RD; Upper right panel - scatter plot 3D QCA-OCT for RD; Lower left panel - scatter plot for 2D QCA-OCT for RefA; Lower right panel – scatter plot 3D QCA-OCT for RefA.

Abbreviations: 2D QCA – two-dimensional quantitative coronary analysis; 3D QCA – three-dimensional quantitative coronary analysis; OCT – optical coherence analysis; RD - reference diameter; RefA - reference lumen area.

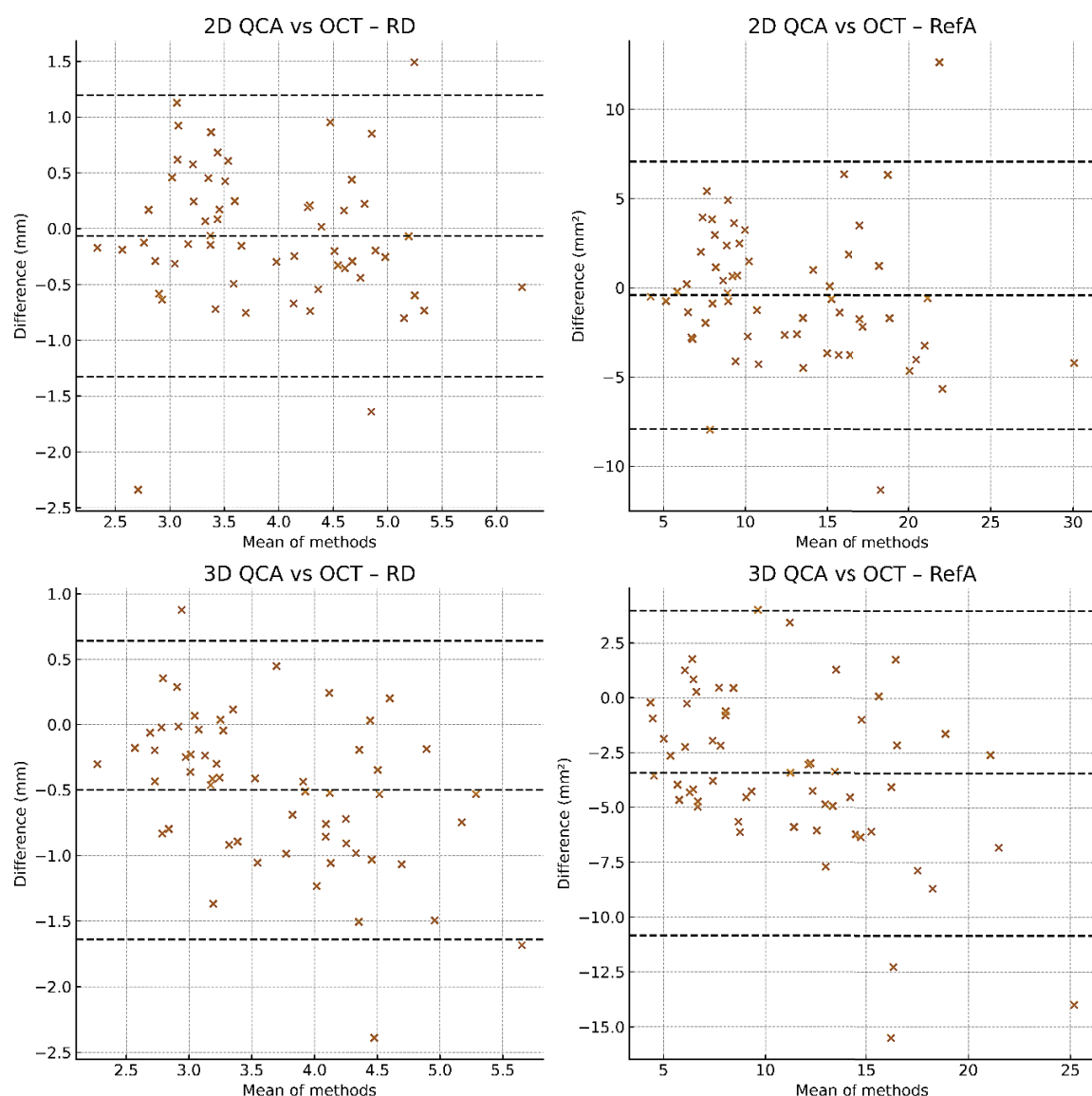


Figure 7. Bland-Altman analysis for 2D-OCT and 3D QCA-OCT for RD and RefA.

Upper left panel – Bland-Altman analysis 2D QCA-OCT for RD; Upper right panel - Bland-Altman analysis 2D QCA-OCT for RefA; Lower left panel - Bland-Altman analysis 3D QCA-OCT for RD; Lower right panel – Bland-Altman analysis 3D QCA-OCT for RefA.

Abbreviations: 2D QCA – two-dimensional quantitative coronary analysis; 3D QCA – three-dimensional quantitative coronary analysis; OCT – optical coherence analysis; RD - reference diameter; RefA - reference lumen area.

ingfully from OCT-derived reference values. 3D QCA demonstrated reduced geometric dispersion compared with 2D QCA; however, clinically relevant variability persisted, underscoring the limitations of angiography-based measurements for precise lumen assessment in LM PCI and supporting the complementary role of IVI.

DISCUSSION

In this study, we systematically compared 2D and 3D quantitative coronary analysis with optical coherence tomography for the assessment of left main coronary lumen dimensions. The main findings can be summarized as follows: first, significant and systematic differences were observed between angiography-based measurements

and OCT across most luminal parameters; second, QCA demonstrated strong associations with OCT, particularly for reference-based and mean lumen metrics; and third, despite these associations, absolute agreement at the individual patient level remained limited, with wide limits of agreement that challenge the clinical interchangeability of these modalities. Notably, 3D QCA consistently reduced geometric dispersion compared with 2D QCA, yet did not fully overcome the limitations inherent to angiography-based assessment in the left main segment.

The RD measured in this study was consistent with those described in previous QCA analyses of LM bifurcation anatomy. Reddy et al. reported QCA-derived RD of 4.50 ± 0.79 mm for LM and 3.45 ± 0.63 mm for LAD (here dMB), while Goel et al. found LM values of 3.89 ± 0.25 mm with LAD measurements of 3.36 ± 0.28 mm

(6, 7). In the present analysis, the LM reference diameter of 4.52 mm closely mirrors Reddy's findings, whereas the LAD reference diameter of 3.22 mm is modestly smaller. This reduction is compatible with long-term post-PCI follow-up measurements, in which late lumen loss (typically around 0.10 mm) contributes to smaller lumen dimensions, particularly in the LAD. The magnitude and direction of the modality differences observed here align well with previously published comparisons of intravascular imaging (IVI) and QCA. Goel et al. and Reddy et al. consistently demonstrated that IVUS-derived diameters exceed QCA-derived measurements in LM and LAD segments; IVUS LM diameters reached 4.33 ± 0.32 mm compared with 3.89 ± 0.25 mm by QCA, while LAD IVUS measurements (3.71 ± 0.60 mm) exceeded QCA values (3.45 ± 0.63 mm). Furthermore, IVUS has been shown to provide larger measurements than OCT, and both exceed histomorphometric references. The measurement hierarchy demonstrated here, with OCT producing the smallest MLD values, QCA systematically underestimating reference dimensions, is consistent with the inter-modality gradient reported across prior intravascular imaging studies, where IVUS exceeds both.

3D QCA was developed to overcome the geometric and projectional limitations of 2D QCA by integrating multiple angiographic views into a three-dimensional reconstruction that better accounts for vessel tortuosity and lesion eccentricity (8–10). Although it shares the resolution constraints of angiography, multiple phantom and stent-validation studies have demonstrated high measurement accuracy, supporting its theoretical advantage in complex coronary anatomy. Comparative imaging literature consistently confirms a modality hierarchy, first described by Tu et al. and later corroborated by Tomaniak et al., in which IVUS yields the largest lumen dimensions, OCT slightly smaller, and 3D QCA the smallest, with IVUS exceeding 3D QCA by 6–14% in diameters and >20% in lumen area, and OCT by 5–10% in diameters and ~16.5% in area (8, 11).

Findings in this study are consistent with prior findings: 3D QCA systematically underestimated OCT, with differences of approximately 13% for RD, 28% for RefA, and 12% for MeanA, mainly attributable to an eccentric lumen geometry (OCT eccentricity ~0.2) and the inherent limitations of diameter-based reconstruction. The most significant discrepancies were observed for refA, expected given that 3D QCA calculates area from at least two diameters, neither of which is guaranteed to represent the actual maximal axis. In contrast, 2D QCA showed markedly fewer deviations (~1.5% in the reference area), likely because all measurements were performed in a caudal projection that naturally aligns with the maximal diameter plane of the LM bifurcation.

Despite these systematic underestimations, 3D QCA demonstrated strong correlations with OCT for key geometric indices in our study ($r \approx 0.78$ – 0.85), consistent

with the conclusions of Tu et al., who reported superior OCT–3D QCA agreement compared with IVUS. Collectively, these findings reinforce that while 3D QCA cannot replace OCT, it provides a meaningfully improved geometric assessment over 2D QCA and offers practical benefit for evaluating complex LM segments when intravascular imaging is unavailable.

Taken together, these findings show that QCA and OCT demonstrate strong associations for lumen and reference-based geometric parameters, while diverging for measurements influenced by extreme vessel sizes, with inter-modality differences comparable in magnitude to those reported in prior QCA–IVUS–OCT studies. (6–8, 12, 13).

CLINICAL IMPLICATIONS

The results of this study have practical implications for the assessment of LM and proximal dMB. While QCA provides reliable information on lumen and reference dimensions, reduced agreement with OCT for minimal and maximal luminal measurements underscores its limitations in characterizing the extremes of lumen geometry. Because slight differences in minimal and maximal lumen dimensions parameters may influence stent sizing and optimization strategies, particularly in LM interventions, intravascular imaging remains the preferred modality for precise evaluation. Nonetheless, the strong correlations observed between RD and RefA indicate a consistent association across modalities, despite the individual variability observed in the Bland–Altman analysis.

STUDY LIMITATIONS

This study has several limitations that should be acknowledged. First, the analysis was restricted to two predefined segments—the left main and distal main branch—without evaluation of the circumflex or additional bifurcation daughter vessels, which limits generalizability to complete bifurcation anatomy. Second, lumen measurements were obtained during long-term post-PCI follow-up, a period in which neointimal proliferation and late lumen loss may influence absolute dimensions; therefore, comparisons with studies based on pre-intervention or acute-phase imaging must be interpreted cautiously. Third, although OCT served as the reference modality, differences in resolution, segmentation algorithms, and frame selection across techniques may have contributed to measurement variability, particularly for parameters defined by maximal luminal contours. Fourth, the sample size, while adequate for paired comparisons and correlation analyses, limits the ability to perform subgroup analyses or adjust for clinical and anatomical covariates. Finally, Bland–Altman plots demonstrated broad limits

of agreement at the individual level, which may affect lesion-level decision-making. The relatively small sample size is a limitation of this study, and further investigations in larger cohorts are warranted.

CONCLUSION

Both 2D and 3D QCA demonstrated significant positive correlations with OCT. However, wide limits of agreement in both modalities confirm that QCA cannot reliably substitute for precise LM lumen sizing with OCT. These findings highlight the value of IVI and support the use of OCT whenever accurate lumen-geometric assessment is required in the LMCA segment.

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Informed consent: Informed consent was obtained from all subjects involved in the study.

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PROCENA DIJAMETRA LUMENA GLAVNOG STABLA LEVE KORONARNE ARTERIJE KVANTITATIVNOM KORONARNOM ANGIOGRAFIJOM I OPTIČKOM KOHERENTNOM TOMOGRAFIJOM

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Sažetak

Uvod: Precizna procena dimenzija lumena glavnog stabla leve koronarne arterije (LM) od ključnog je značaja tokom perkutane koronarne intervencije (PCI). Kvantitativna koronarna analiza (QCA) se rutinski koristi u kliničkoj praksi, dok stepen njenog slaganja sa intravaskularnim modalitetima visoke rezolucije, poput optičke koherentne tomografije (OCT), u LM segmentu nije u potpunosti razjašnjen.

Metode: U studiju je uključeno 30 bolesnika kod kojih je tokom praćenja sprovedena angiografija i OCT nakon PCI na LM. Analizirano je osam odgovarajućih parametara lumena dobijenih pomoću 2D QCA, 3D QCA i OCT. Razlike između metoda procenjene su Friedmanovim testom, povezanost analizirana je Pearsonovom korelacijom, dok je slaganje metoda ispitivano Bland-Altman analizom.

Rezultati: Uočene su značajne razlike između modaliteta za većinu analiziranih parametara lumena. QCA je pokazala snažnu povezanost sa OCT za referentne i srednje vrednosti lumena, dok je povezanost bila slabija za ekstremne (minimalne i maksimalne) dimenzije. Bland-Altman analiza je pokazala mala prosečna odstupanja između QCA i OCT, ali uz široke granice slaganja, što ukazuje na izraženu interindividualnu varijabilnost uprkos konzistentnosti na nivou grupe.

Zaključak: QCA obezbeđuje relativno konzistentne procene referentnih i srednjih dimenzija lumena u LM segmentu, ali pokazuje ograničenu korelaciju sa OCT, naročito za ekstremne vrednosti. Uprkos snažnoj povezanosti, individualna varijabilnost merenja ostaje značajna, što potvrđuje OCT kao preferentni modalitet za preciznu procenu lumena, izbor veličine stenta i optimizaciju PCI na LM.

Ključne reči: optička koherentna tomografija, kvantitativna koronarna angiografija, glavno stablo leve koronarne arterije, korelaciona analiza, veličina lumena

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ORIGINAL ARTICLE

Distant metastasis – free interval and overall survival in breast sarcoma: a single-institution retrospective study

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Summary

Introduction: Breast sarcomas (BS) are rare malignant tumors with aggressive behavior and limited evidence guiding outcome assessment. Data on distant metastasis-free interval (DMFI) in this population are scarce. This study aimed to describe oncologic outcomes and clinicopathologic characteristics in patients with BS treated with curative intent.

Methods: Patients with primary or secondary breast sarcoma treated at a single tertiary cancer center between 2013 and 2025 were retrospectively analyzed. Patients with metastatic disease at diagnosis were excluded. DMFI was the primary endpoint, and OS was the endpoint. Survival outcomes were estimated using Kaplan–Meier methodology and presented descriptively.

Results: Twenty-six patients met the inclusion criteria. Angiosarcoma was the most common histologic subtype, predominantly occurring as secondary disease after prior breast radiotherapy. Median DMFI was not reached during follow-up, with 71.5% of patients remaining metastasis-free at 3 years, whereas median OS was 31 months with a 3-year OS of 46.9%. Patients with higher pathological T stage, larger tumors, tumor necrosis, younger age, and narrower resection margins tended to demonstrate shorter DMFI. Differences in overall survival were most pronounced according to resection margin status.

Conclusions: Tumor burden-related factors, tumor necrosis, and surgical margin width showed clinically relevant differences in metastasis-related outcomes and survival in breast sarcoma. Adequate surgical resection remains critical in this rare and heterogeneous disease.

Keywords: breast sarcoma, distant metastasis-free interval, overall survival, surgical margins, angiosarcoma

INTRODUCTION

Breast sarcomas (BS) are rare malignant tumors of mesenchymal origin, accounting for less than 0.1% of all breast malignancies (1, 2, 3). They are characterized by aggressive biological behavior, limited responsiveness to adjuvant therapies, and high propensity for local recurrence and distant metastatic spread, which largely determines long-term outcomes. Unlike epithelial breast cancers, breast sarcomas typically disseminate hematogenously, and distant metastases represent a major cause of disease-related mortality. (4, 5). Breast sarcomas may be classified as primary or secondary. Primary tumors arise *de novo* from the breast or associated soft tissues. Sometimes they may be associated with hereditary cancer syndromes, including Li-Fraumeni syndrome, familial adenomatous polyposis, neurofibromatosis type 1, and hereditary retinoblastoma (6). Secondary breast sarcomas most often develop as a rare late complication of postoperative breast irradiation or in the setting of chronic lymphedema, with radiation-associated angiosarcoma representing the most common histologic subtype (7, 8).

Complete surgical excision with negative margins is the mainstay of treatment for BS, whereas the roles of adjuvant radiotherapy and systemic therapy remain incompletely defined (1, 4, 9,10). Evidence supporting these practices primarily derives from small retrospective series and institutional reports (11, 12).

Given these limitations, institutional analyses are essential to improve understanding of disease behavior and prognostic factors. This study aimed to describe oncologic outcomes and to characterize clinicopathologic features in relation to distant metastasis-free interval (DMFI) and overall survival (OS) in patients with BS treated with curative intent.

MATERIALS AND METHODS

Patients and data

This retrospective cohort study was conducted on patients treated at a tertiary cancer center between January 2013 and September 2025. Eligible patients had a histopathologically confirmed diagnosis of primary or secondary BS arising within the breast or breast-associated soft tissues, underwent curative-intent surgical treatment at our institution, and had no evidence of distant metastases at diagnosis. Patients were required to have complete clinical, pathological, treatment, and follow-up data available for analysis. Patients with metastatic disease at presentation, palliative-intent treatment, primary treatment performed outside our institution, isolated chest wall sarcomas without breast involvement, or incomplete medical records were excluded from the study. Clinical, pathological, treatment, and follow-up data, including

patient age, tumor characteristics, surgical procedures, radiotherapy and systemic therapy parameters were retrieved from medical records and pathology reports. This study was reviewed and approved by the Ethics Committee of the Institute for Oncology and Radiology of Serbia, approval number 01-1/2025/3282.

Treatment

All patients included in this analysis underwent complete diagnostic evaluation and oncologic treatment at our institution. Surgical management consisted of wide local excision or mastectomy, with axillary lymph node surgery performed when clinically indicated. Decisions regarding the overall treatment strategy, including the extent of surgery and the use of adjuvant radiotherapy and/or systemic therapy, were made at a multidisciplinary breast tumor board meeting.

Follow-up and long-term outcomes

Patients were followed every 3 months during the first postoperative year, every 6 months from years 2 to 5, and annually thereafter. The primary study endpoint was DMFI, defined as the time from completion of primary treatment to the first clinically or radiologically confirmed distant metastasis. Overall survival was a secondary endpoint and was defined as the time from completion of primary treatment to death from any cause.

Statistical analysis

Categorical variables were presented as absolute and relative frequencies, whereas continuous variables were summarized using descriptive statistics, including mean, median, standard deviation (SD), and range. Survival outcomes (DMFI and OS) were estimated using the Kaplan-Meier method and presented as median survival times and survival rates at selected time points. Due to the limited sample size, no formal comparative or multivariable analyses were performed, and the results are presented descriptively. All statistical analyses were conducted using R software (version 4.3.1 (2023-06-16 ucrt)—“Beagle Scouts”; Copyright (C) 2023 The R Foundation for Statistical Computing; Platform: x86_64-w64-mingw32/x64 (64-bit)) (available at: www.r-project.org; downloaded: 21 August 2023).

RESULTS

Patients and treatment

Between January 2013 and September 2025, 26 patients treated at our institution met the inclusion criteria for the present study. Patient's clinicopathological and treatment

Table 1. Clinicopathological and treatment characteristics of the study cohort.

PATIENT CHARACTERISTICS		PATIENT CHARACTERISTICS	
Age (years)		Enlarged LN	
Mean (SD)	61.3 (12.2)	Yes	1 (3.9%)
Median (Range)	60.5 (35-92)	No	25 (96.1%)
CCI		Follow up (months)	
Mean (SD)	1.69 (1.49)	Mean (SD)	28.2 (28.9)
Median (Range)	2 (0-6)	Median (Range)	20 (2-113)
Previous BC		TREATMENT CHARACTERISTICS	
Yes	16 (61.5%)		
No	10 (38.5%)	Breast surgery	
Previous RT		WLE	
Yes	14 (53.8%)	3 (11.5%)	
No	12 (46.2%)	Mastectomy	
Laterality		23 (88.5%)	
Right	17 (65.4%)	Axillary dissection	
Left	9 (34.6%)	Yes	
CTS (mm)		4 (15.4%)	
Mean (SD)	74.1 (60)	No	
Median (Range)	60 (15-300)	22 (84.6%)	
Localization		Reconstruction	
CQ	10 (38.5%)	Yes	
ULQ	4 (15.4%)	6 (23.1%)	
LLQ	4 (15.4%)	No	
LMQ	3 (11.5%)	20 (76.9%)	
UMQ	5 (19.2%)	Adjuvant CT	
Total		Yes	
	26 (100%)	3 (11.5%)	
		No	
		23 (88.5%)	
		Adjuvant RT	
		Yes	
		4 (15.4%)	
		No	
		22 (84.6%)	
		Total	
		26 (100%)	

Abbreviations: SD – standard deviation; CCI – Charlson comorbidity index; BC – breast cancer; RT – radiotherapy; CTS – clinical tumor size; mm – millimeter; CQ – central quadrant; ULQ – upper lateral quadrant; LLQ – lower lateral quadrant; LMQ – lower medial quadrant; UMQ – upper medial quadrant; LN – lymph node; WLE – wide local excision; CT – chemotherapy.

data are shown in **Table 1**. All patients were females. The cohort consisted predominantly of older patients, many with a history of prior breast cancer and radiotherapy, consistent with secondary BS. Tumors were generally large and clinically node-negative at presentation. Surgical treatment was primarily mastectomy, with axillary procedures used selectively, and adjuvant therapies applied on an individualized basis.

Postoperative histopathological characteristics, staging, and long-term outcomes

Postoperative histopathological analysis revealed a predominance of high-grade tumors, with angiosarcoma representing the most frequent histologic subtype. Out of 15 patients with angiosarcoma, 13 (86.7%) received previous radiotherapy for breast cancer treatment. On the other hand, only one patient in our cohort developed a tumor other than angiosarcoma after RT (undifferentiated pleomorphic sarcoma). Tumors were generally large and exhibited a high proliferative activity, with tumor necrosis commonly observed. Detailed histopathological characteristics are summarized in **Table 2**. Most patients

achieved microscopically negative (R0) resection, although a small proportion had positive margins (n=2).

During follow-up, eight patients experienced distant metastatic progression, while death was recorded in twelve patients. Kaplan–Meier curves for DMFI and OS are shown in **Figure 1**. Median DMFI was not reached during follow-up (95% CI, >39 months), with 71.5% (95% CI: 55.6%-92.1%) patients being metastasis-free after 3 years. The median overall survival was 31 months (95% CI, >20 months) with 3-year OS of 46.9% (95% CI: 28.6%-76.8%).

Clinicopathological characteristics according to outcomes

Continuous variables were explored descriptively across outcome groups. Due to the limited number of events, no formal threshold determination was considered reliable, and results are presented descriptively.

Kaplan–Meier curves indicated differences in DMFI across several clinicopathological subgroups, including tumor size, pathological T stage, tumor necrosis, patient age, and resection margin width. Differences in OS were

Table 2. Histopathological characteristics of the study cohort

HISTOPATHOLOGICAL CHARACTERISTICS			
Sarcoma type		PTS (mm)	
Angiosarcoma	15 (57.7%)	Mean (SD)	84.4 (62.4)
Other	11 (42.3%)	Median (Range)	67.5 (20-300)
Ki67 (%)		pT	
Mean (SD)	53.3 (24)	T1	12 (46.1%)
Median (Range)	55 (5-95)	≥T2	14 (53.9%)
Grade		Resection margin (mm)	
I	5 (19.2%)	Mean (SD)	26.8 (14.9)
II	10 (38.5%)	Median (Range)	25 (0-57)
III	11 (42.3%)	R status	
Tumor necrosis		R0	24 (92.3%)
Yes	14 (53.8%)	R1	2 (7.7%)
No	12 (46.2%)	Total	
			26 (100%)

Abbreviations: SD – standard deviation; PTS – pathological tumor size; pT – pathological T stage; mm – millimeter.

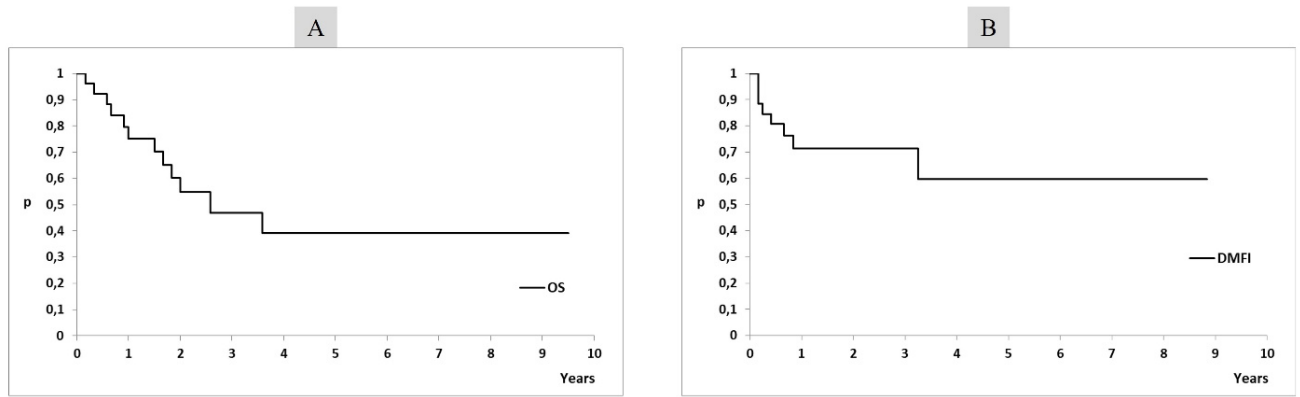


Figure 1. A. Kaplan–Meier estimate of overall survival of the entire study cohort. B. Kaplan–Meier estimate of distant metastasis-free interval of the entire study cohort.

observed across subgroups, particularly according to resection margin status. This parameter demonstrated a similar pattern across both DMFI and OS curves.

DISCUSSION

In this single-institution retrospective cohort of BS patients treated with curative intent, we observed a disease profile characterized by large tumors at presentation, frequent angiosarcoma, often in the context of prior breast cancer radiotherapy. Tumor size at presentation in our cohort exceeded that reported in comparable series (2, 11), while age at diagnosis was consistent with previously published data (2, 11, 13, 14).

This is, to our knowledge, the only study to analyze DMFI in BS patients. The discrepancy between 3-year overall survival and distant metastasis-free rates reflects deaths occurring without documented metastatic disease, most commonly due to aggressive local progression, treatment-related morbidity, or competing comorbidities in older patients. In our cohort, variation in DMFI

appeared to be associated with tumor burden-related parameters (pT stage and tumor size), resection margins, tumor necrosis, and patient age. Fields et al. reported increased risk for death, local recurrence, and development of metastases in patients with tumors larger than 5cm in diameter, which is comparable to findings in our cohort (4). Similar results were published by Adem et al in the Mayo Clinic series and literature review (1). These observations are consistent with the broader soft tissue sarcoma literature, which reports that tumor size, necrosis, and features of aggressive biology are commonly associated with metastatic risk and survival across histologic subtypes (15, 16, 17). Because BS series are typically small and biologically heterogeneous, DMFI may provide particularly informative insight into systemic disease behavior. In contrast, overall survival may be influenced by competing risks, salvage treatments, and comorbidities, especially in older patient populations.

In contrast, in our cohort, OS differed primarily by resection margin width, with a consistent pattern across both DMFI and OS. Positive resection margins in other cohorts were associated with significantly worse treat-

ment outcomes (14). In our cohort, only two patients had positive resection margins, with time to death of 4 and 7 months, compared to a median OS of 43 months in R0 patients. The small number of R1 patients precluded further analysis. This supports the longstanding principle that complete surgical resection with negative margins is the cornerstone of sarcoma treatment, emphasized in major guidelines and consistently observed in BS series (2, 9, 12, 18). Although BS predominantly disseminates hematogenously, surgical margin status remains a meaningful surrogate for oncologic clearance, particularly in infiltrative histologies such as angiosarcoma, where microscopic residual disease may drive early recurrence and systemic relapse (19, 20, 21).

Interestingly, older age was accompanied by a parallel, although not statistically significant, trend toward longer OS in our cohort. While counterintuitive, this observation likely reflects the complex, context-dependent influence of age on sarcoma outcomes rather than a direct biological advantage. Given the limited sample size and number of events, these results should be interpreted cautiously and should not be overinterpreted. Notably, prior breast and soft tissue sarcoma studies have not consistently demonstrated a similar association between older age and improved metastasis-related or survival outcomes.

The inclusion of both primary and secondary BS in the present analysis warrants specific consideration. In our cohort, 86.7% of angiosarcomas occurred as secondary BS; however, no clear differences in DMFI or OS were apparent between angiosarcoma and other sarcoma subtypes. This finding should be interpreted cautiously, given the limited sample size and number of events. Secondary BS, most commonly radiation-associated angiosarcomas, are recognized as a biologically distinct subgroup, often characterized in the literature as having higher rates of local recurrence and poorer survival than primary sarcomas (20, 22, 23). Nevertheless, prior institutional and population-based studies have frequently analyzed primary and secondary BS together, reflecting the rarity of the disease and the shared clinical challenge of managing aggressive mesenchymal tumors arising in the breast (3, 24). While this approach increases biological heterogeneity, it allows for clinically meaningful evaluation of outcome patterns and prognostic factors across the broader spectrum of BS. Importantly, tumor burden and adequacy of surgical resection, factors identified in the

present study, have been consistently reported as dominant determinants of outcome in both primary and radiation-associated BS, supporting the relevance of combined analyses in exploratory outcome research (18, 20).

This study is limited by its retrospective, single-center design and small sample size, which restricts generalizability. The low number of distant metastatic events precluded reliable multivariable modeling; therefore, the analyses were interpreted descriptively. The inclusion of both primary and secondary BS further increases biological heterogeneity, a challenge inherent to studies of rare tumors. In addition, the low number of distant metastatic events limited the ability to perform robust multivariable analyses.

In conclusion, shorter DMFI tended to occur in patients with higher tumor burden–related factors, tumor necrosis, and older age. At the same time, resection margin width showed a consistent relationship with both DMFI and OS. These findings indicate that metastasis-related outcomes may provide complementary information to overall survival regarding systemic disease behavior in this rare and heterogeneous population. Larger, multi-institutional studies are needed to explore these observations further and better define prognostic factors in BS.

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INTERVAL BEZ UDALJENIH METASTAZA I UKUPNO PREŽIVLJAVANJE KOD SARKOMA DOJKE: RETROSPEKTIVNA STUDIJA U TERCIJARNOM CENTRU

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Sažetak

Uvod: Sarkomi dojke (SD) predstavljaju retke, agresivne maligne tumore sa ograničenim dokazima o dugoročnim ishodima lečenja. Cilj ove studije bio je da opiše onkološke ishode i ispita kliničkopatološke faktore povezane sa intervalom bez udaljenih metastaza (DMFI) i ukupnim preživljavanjem (OS) kod pacijenata lečenih sa kurativnom namerom.

Metode: Retrospektivno su analizirani pacijenti sa primarnim ili sekundarnim sarkomom dojke lečeni u jednom tercijarnom onkološkom centru u periodu od 2013. do 2025. godine. Pacijenti sa metastatskom bolešću u trenutku dijagnoze bili su isključeni. DMFI je definisan kao primarni, a OS kao sekundarni ishod. Preživljavanje je procenjivano Kaplan–Majerovom metodom i prikazano deskriptivno.

Rezultati: Ukupno dvadeset šest pacijenata ispunilo je kriterijume uključenja u studiju. Angiosarkom je bio najčešći histološki podtip, uglavnom kao sekundarni tumor nakon prethodne radioterapije dojke. Medijana DMFI nije dostignuta, pri čemu je 71,5% pacijenata bilo bez udaljenih metastaza nakon tri godine, dok je medijana OS iznosila 31 mesec uz trogodišnje OS od 46,9%. Kraći DMFI je tendenciozno uočen kod pacijenata sa višim patološkim T stadijumom, većom veličinom tumora, prisustvom tumorske nekroze, mlađim uzrastom i užim resekcionim marginama. Razlike u OS bile su najizraženije u odnosu na status resekcionih margina.

Zaključak: Tumorsko opterećenje, nekroza tumora i starost bili su povezani sa DMFI, dok je jedino širina hirurških margina bila povezana i sa DMFI i sa OS, naglašavajući značaj adekvatne hirurške resekcije SD.

Ključne reči: sarkom dojke, interval bez udaljenih metastaza, ukupno preživljavanje, hirurške margine, angiosarkom

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REVIEW ARTICLE

Intravenous iron therapy in heart failure with reduced ejection fraction: evidence from recent trials and meta-analyses

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Summary

Anemia is a frequently observed comorbidity in patients with heart failure (HF) and it contributes to increased morbidity and mortality. Even though the etiology of anemia in HF patients is polymorphic, chronic inflammation and iron deficiency (ID) are reported as the most frequent underlying causes. Anemia is a strong prognostic marker in both acute and chronic HF, while ID, even in the absence of anemia, has a negative impact on patients' symptoms, functional capacity, and quality of life (QOL). In chronic HF, functional ID is often present due to impaired iron utilization, but it may coexist with absolute ID, where iron stores are depleted.

Intravenous (IV) iron therapy has been shown to have a beneficial effect on improving functional status, reducing symptoms, and lowering the rate of hospital readmissions. This has led to its inclusion in the latest guidelines of the European Society of Cardiology (ESC) as a recommended treatment for symptomatic patients with heart failure with reduced ejection fraction (HFrEF). Several studies have also described a consistent trend toward reduced mortality.

The aim of this paper is to summarize current knowledge on the importance of ID and the therapeutic effects of iron supplementation in HFrEF patients. Timely recognition and correction of ID should be considered a cornerstone of individualized HFrEF management.

Keywords: heart failure, intravenous iron, iron deficiency, anemia



INTRODUCTION

Heart failure (HF) is a multifaceted clinical syndrome characterized by the heart's inability to adequately deliver oxygenated blood to peripheral tissues, leading to tissue hypoxia. Approximately 2% of the global population is affected, with rising incidence and prevalence being driven by an aging population, increasing comorbidities, and improved management of multiple cardiovascular diseases. The lifetime risk of HF has increased to 24%, which means that approximately one in four individuals will develop HF during their lifetime (1).

The latest major epidemiological research, HF STATS 2024, published in January 2025, indicates that by 2050, the number of affected individuals could increase to 11.4 million. Subsequently, due to these rising numbers, HF-related hospitalizations and mortality are also increasing (2).

One of the most common comorbidities of HF is anemia, which has been associated with reduced functional capacity, increased hospitalization, and higher mortality rates (3).

Its prevalence in HF ranges from 10% to 50%, depending on the population being studied, the diagnostic criteria for HF, and the presence of anemia. Using the World Health Organization (WHO) definition, hemoglobin (Hb) levels below <13 g/dL in men and <12 g/dL in women are sufficient for diagnosis of anemia. Iron deficiency (ID), the most frequent underlying cause of anemia in HF, may be present even in the absence of anemia. It is diagnosed if serum ferritin levels are <100 µg/L or ferritin levels are 100–299 µg/L and the saturation of transferrin (TSAT) is <20% (4).

According to the latest European Society of Cardiology (ESC) guidelines for HF management, intravenous (IV) iron repletion is a class I recommendation in symptomatic HFrEF patients (1, 4).

The complex interaction between anemia and HF has been observed in many trials. The underlying mechanisms of anemia are diverse and are closely linked to the pathophysiology of HF. Anemia directly restricts oxygen delivery to peripheral tissues, and HF is responsible for various mechanisms like chronic inflammation, neuro-hormonal activation, iron dysregulation, and hemodilution. Low-grade and chronic inflammation is most significant in the development of anemia. Pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β), promote the production of hepcidin, a liver-derived peptide hormone, that is the main driver of iron metabolism regulation. Hepcidin binds to ferroportin (FPN), the iron exporter in enterocytes, macrophages, and hepatocytes. At higher levels of hepcidin, the iron export into blood is decreased, resulting in functional ID. In chronic diseases, such as HF, functional ID can coexist with absolute ID, where iron stores are depleted. As a result of chronic hy-

poperfusion and reduced cardiac output, the sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) are chronically activated, leading to blunted erythropoietin (EPO) (3, 4).

Besides inflammatory mechanisms, progressive left ventricular remodeling after acute myocardial infarction (AMI) is still the main etiological factor of HFrEF. It increases myocardial wall stress, oxygen consumption, and reduces perfusion of the subendocardial tissue. A short early filling deceleration time on the first day after AMI is shown to be an independent predictor of adverse left ventricular remodeling and increased long-term cardiac mortality. This mechanism contributes to further deterioration of HF. It may also promote anemia through impaired oxygen delivery and increased metabolic demands (5-7).

Several studies have demonstrated that ID is present in approximately 49–82% of patients with AMI, even in the absence of anemia, and it has been linked to impaired recovery, ST segment resolution, adverse ventricular remodeling, and increased long-term morbidity and mortality. Despite its clinical relevance, iron status testing is still rarely performed in routine care of AMI patients (8-10).

Another HF-related mechanism is hemodilution, an expansion of plasma volume, which leads to relative anemia. It causes overestimation of the severity of anemia, resulting in inadequate therapeutic intervention. Hemodilution also leads to increased cardiac workload, and greater blood volume must be pumped out by an already compromised heart, leading to *circulus vitiosus* (11, 12).

Serum ferritin is commonly elevated during AMI due to its role as an acute-phase reactant. Based on recent studies, it has been proposed that the definition of ID in chronic HF should rely exclusively on low TSAT (13, 14). In the context of negative outcomes and higher mortality, early identification of patients who are at high risk for developing HFrEF after AMI allows for prompt implementation of targeted therapy. In addition to the four foundational pillars (angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor-neprilysin inhibitor (ARNI), beta-blockers, sodium glucose-cotransporter 2 (SGLT2) inhibitors, and mineralocorticoid receptor antagonists (MRAs), this approach also includes recognizing patients who could benefit from early iron supplementation (15).

The purpose of this review is to summarize previous knowledge on the use, efficacy, and safety of IV iron in patients with HF, highlighting its beneficial effects, while also addressing its limitations with respect to mortality.

METHODS

The literature search was performed using the PubMed database, focusing on studies published in the last five years. The search included studies written in English and conducted only on human subjects. A few studies

published outside this timeframe were also included due to their scientific relevance. The following keywords were used: “heart failure”, “iron deficiency”, “intravenous iron”, and “anemia”. Priority was given to RCTs, systematic reviews, and meta-analyses. Reference lists of the selected articles were also reviewed to identify any further relevant publications.

LARGE-SCALE CLINICAL TRIALS OF IV IRON THERAPY IN HFrEF

The majority of randomized control studies (RCTs) have focused on the effects of IV iron therapy given the established link between ID and poor clinical outcomes in HF.

The FAIR-HF trial is the first large RCT to explore IV iron supplementation using ferric carboxymaltose (FCM) in HFrEF patients. The primary endpoints were the self-reported Patient Global Assessment (PGA) and the New York Heart Association (NYHA) functional class. Secondary endpoints included the six-minute walk distance (6MWT) and health-related quality of life (QOL) questionnaire. Half of the patients having FCM showed an improvement in PGA compared to less than 30% in the placebo group, which was statistically significant. There was an increased QOL in the IV iron group and enhanced exercise capacity by 6MWT compared to the control group. A consistent improvement in 6MWT and QOL was registered just 4 weeks after the treatment and persisted at 12 and 24 weeks. This trial showed an improvement of 35 ± 8 m in the FCM group (all p values were < 0.001). The FAIR HF trial, published in 2009, served as the gateway that enabled IV FCM to be included in the ESC recommendations in 2016 (16, 17).

The extension of this trial was CONFIRM-HF, which focused on the long-term effects of IV FCM in HF patients with ID. The primary outcome was only the improvement in 6MWT at week 24. After 24 weeks, there was an increase in 6MWT distance by 18 ± 8 m in the FCM group, while it decreased by 16 ± 8 m in the placebo group. There was a consistent and sustained improvement in 6MWT over time and across all subgroups. According to Professor Ponikowski, the chief investigator, benefits like this have only been observed with cardiac resynchronization therapy. There was a sustained improvement in functional capacity over 52 weeks, reduced hospitalization rate for 61% due to worsening of HF, better symptom control, and NYHA functional class improvement. The patients with diabetes and impaired renal function responded better to IV iron therapy (18).

In the FAIR-HF trial, the dosage of FCM was calculated using the Ganzoni equation, based on the patient's weight, target, and current Hb values. This approach was tailored to patients' individual needs, but complicated for routine clinical practice. The dosing frequency was initially weekly until repletion was achieved, and

then maintained every 4 weeks. On the other hand, the CONFIRM-HF trial used a simpler dosing regimen, starting with a single high dose of FCM, followed by additional smaller doses administered only as needed, based on follow up measurements of iron parameters. This simplified dosing strategy enabled efficient correction of ID in everyday clinical practice. More than 75% of the patients required no more than two doses of FCM for correction and maintenance of iron levels. Irrespective of dosage regimen an improvement in NYHA functional class was observed in both trials after 24 weeks of treatment (16, 18).

The AFFIRM-HF trial conducted from 2017 to 2020 specifically assessed IV iron in patients hospitalized for acute HF (AHF) decompensation. The main rationale for this study was the fact that approximately 80% of patients with acute HF have ID (19).

Also, de novo AHF and acute decompensation of chronic HF represent clinically and pathophysiological distinct entities, but both are associated with high intra-hospital and one-year mortality. And even though they fall under the same clinical syndrome, these two forms differ in severity of clinical presentation, laboratory findings, and treatment response (20).

Results of AFFIRM-HF showed 26% reduced risk of HF rehospitalization rate in the IV iron group, with no significant reduction in cardiovascular mortality. The AFFIRM-HF provided strong evidence for IV iron therapy as a strategy for short-term clinical improvement. However, the relatively short follow-up period of 52 weeks and the lack of mortality benefit enhanced the need for further long-term studies. Benefits were independent of baseline Hb levels, reinforcing the concept that ID itself, rather than anemia alone, could be a key therapeutic target in HF (19).

The IRONMAN trial conducted from 2016 to 2021 was the first large RCT that evaluated the long-term effects and safety of IV iron gathering information from 70 UK hospitals. The trial used IV ferric derisomaltose, rather than FCM, providing insight into the differences between IV iron formulations in HF. Ferric derisomaltose can be administered in high doses via infusion. It is already commonly used in other medical specialties, including nephrology, gastroenterology, gynecology, and in the preoperative setting. The primary endpoint was a composite of recurrent HF hospital admissions and cardiovascular death. Although the trial itself did not reach statistical significance for this outcome ($p=0.07$), when interpreted alongside the AFFIRM-AHF trial (which used a different iron formulation) the overall conclusion supports the clinical benefit of this type of treatment in HFrEF patients. Because of the impact of the COVID-19 pandemic, the chief investigator professor Karla conducted a pre-specified subgroup analysis of 1063 patients enrolled prior to March 31, 2020, before the first lockdown. In this analysis, IV iron therapy was associated

with a 24% relative risk reduction (RRR) in the primary outcome compared to usual treatment, reaching its statistical significance ($p=0.047$). Additionally, in a post hoc analysis where follow-up was censored at one year to allow direct comparison with AFFIRM-AHF, the observed treatment effect was even more pronounced (21).

The latest multicenter RCT, the FAIR-HF2 trial, challenged previously established findings on HF hospitalization rates. It is essential to note that this trial simultaneously evaluated three distinct primary endpoints, in contrast to previous trials that focused on a composite primary endpoint. As a consequence, it required statistical correction of p-values, using the Hochberg procedure, to avoid the type one error rate and false positive results. This method is more rigorous, but it also reduces the statistical power, making it harder to achieve statistical significance. In contrast, composite endpoints may mask differential effects on individual outcomes. There was a consistent RRR of approximately 21% in all three primary endpoints, but none of them reached statistical significance. The first primary endpoint was time to cardiovascular death or first hospitalization due to HF. Although the IV iron group showed a lower event rate with a raw p-value of 0.04, this difference was no longer statistically significant after correcting it. The second primary endpoint was total hospitalizations for HF, which also showed no statistically significant difference between groups. The third primary outcome targeted a specific subgroup of patients with TSAT below 20%, as earlier studies had suggested they might have the most benefit from IV iron. In the 1105 patients included, 70% of them met this criterion; yet, the observed hazard ratio of 0.74 ($p = 0.07$) did not achieve statistical significance.

Notably, in the FAIR HF2 trial, sex-specific differences emerged. Male patients showed more favorable outcomes, while female patients exhibited no significant reduction in cardiovascular events or hospitalizations. This may be a reflection of differences in iron metabolism, hormonal regulation, or even underrepresentation in clinical trials. Similarly, no significant differences were observed between ischemic and non-ischemic etiologies

of HF, suggesting a broadly consistent therapeutic effect across subtypes. Also, this trial spanned the COVID-19 pandemic, which we believe may have influenced patient recruitment, care delivery, and outcome rates. This could partly explain the neutral findings, as similar disruptions were observed in other cardiovascular trials conducted during the same period. Despite these limitations, the FAIR-HF2 confirms the benefits of IV-iron therapy on quality of life and patient self-reported health status (22).

Over a decade later from publishing FAIR HF and IV iron entering the ESC recommendations, the biggest HEART-FID trial sought to resolve this therapy-mortality evidence gap by evaluating the impact of IV FCM on a hierarchical composite endpoint including all-cause mortality, HF hospitalizations, and functional status. Despite a larger sample size and more rigorous methodology, this trial failed to show a statistically significant reduction in mortality, although it confirmed benefits mentioned in previous trials. These findings highlight that while IV iron therapy plays a crucial role in symptom management and reducing morbidity, its effect on survival remains uncertain, emphasizing the need for further large-scale research (23).

The summary of all studies is presented in **Table 1**.

This table summarizes the main findings of key randomized controlled trials investigating intravenous iron therapy in patients with HfrEF, emphasizing improvements in functional capacity and hospitalization rate.

In the meta-analysis by Graham et al., which included over 3000 patients, IV iron therapy was associated with a 25% RRR in the primary composite outcome of recurrent HF hospitalizations and cardiovascular mortality. A reduction in HF hospitalizations predominantly drove this result, while the impact on mortality remained inconsistent. Implementing IV iron in HF treatment reduced cardiovascular death by 14% and overall mortality by 7% but the difference did not reach statistical significance. Subgroup analysis revealed that patients with ischemic heart disease appeared to derive a greater benefit from IV iron (odds ratio 0.84) compared to those with non-ischemic etiologies (odds ratio 0.98), although this difference was not statistically significant ($p = 0.28$). Similarly, patients with TSAT

Table 1. Summary of key trials investigating intravenous iron in heart failure

Study	Year	Number of patients	Primary outcome	Main result
FAIR-HF ¹⁶	2009	459	PGA and NYHA class improvement	PGA, NYHA class, 6MWT and QOL improvement
CONFIRM-HF ¹⁸	2014	304	6MWT	Improved functional capacity, NYHA class, reduced hospitalizations for 61%
AFFIRM-HF ¹⁹	2020	1132	HHF or CV death	Reduction in rehospitalizations
IRONMAN ²¹	2023	1137	HHF or CV death	Reduction in hospitalizations; mortality showed a trend toward reduction
FAIR-HF2 ²²	2024	1105	3 distinct primary outcomes	No significant difference in neither hospitalization nor mortality
HEART-FID ²³	2024	3065	Hierarchical composite outcome	Functional improvement and fewer hospitalizations; mortality without statistical difference

PGA, Patient Global Assessment; NYHA, New York Heart Association functional class; HF, heart failure; CV, cardiovascular; 6MWT, 6-minute walking test; HHF, heart failure hospitalization; QOL, quality of life

Table 2. Major recent meta-analyses on intravenous iron therapy in heart failure

Study	Year	Journal	Patients included	Main Focus	Key Findings
Graham et al. ²⁴	2023	European Journal of Heart Failure	3373	Recurrent HHF, CV death and all-cause mortality	IV iron reduces HHF especially in ischemic HF and patients with lower TSAT
Ahmed et al. ²⁵	2025	ESC Heart Failure	6651	Composite outcome (first HHF and CV death)	Reduced incidence of the composite outcome
Anker et al. ²⁷	2025	Nature Medicine	7175	Composite outcomes, functional parameters	IV iron reduces hospitalizations; CV death

HF, heart failure, HHF, heart failure hospitalization, CV, cardiovascular, ESC, European Society of Cardiology, IV intravenous, TSAT, saturation of transferrin

<20% experienced more pronounced benefit (odds ratio 0.67) than those with higher values of TSAT. Overall, the heterogeneity of data across the meta-analysis was low (24).

The meta-analysis conducted by Ahmed et al. included 14 RCTs and a total of 6,651 patients. Importantly, their meta-analysis included the most recent large trial HEART-FID (25).

There was a consistent trend toward reduction in overall mortality, 1-year and overall cardiovascular mortality, although no statistical significance was observed. High heterogeneity was registered when analyzing NYHA class, 6MWT, and left ventricular ejection fraction (LVEF). To address this, the authors conducted “leave-one-out” sensitivity analyses, identifying outlier studies that contributed most to variability. Excluding the trial undertaken by Dhoot et al., heterogeneity in LVEF analysis was reduced to 0%. Those results revealed a significant improvement in LVEF for 7.02% ($p < 0.00001$). The reason for that could be that the study by Dhoot et al. included a small sample size ($n = 70$), Indian population, and a short follow-up duration of only 12 weeks (26).

Additionally, by removing the trial by Kalra et al. heterogeneity when analyzing the 6MWT distance, the reduction was increased to 72%, demonstrating a significant benefit (MD = 30.25 m, $p = 0.03$). However, NYHA class remained highly heterogeneous despite exclusion strategies. These findings confirm the rationale and patient-reported benefits observed in prior studies such as CONFIRM-HF and FAIR-HF. This could be attributed to the variation in follow-up length and patient characteristics among the included studies (25).

At the same time when the FAIR HF2 trial results were released, Anker et al. published a Bayesian meta-analysis that pooled data from six major RCTs. The meta-analysis included more than 7,100 patients and showed a 28% reduction in recurrent HF hospitalizations and cardiovascular death within 12 months ($p = 0.010$), as well as a 13–18% reduction in cardiovascular mortality, confirming the therapeutic value of iron supplementation in HFrEF. These findings strengthen and support the idea that the inconsistency of dosing beyond the first year may have impacted outcomes in the FAIR HF2 trial (27).

Major recent meta analyses on IV iron therapy in HF are summarized in [Table 2](#).

This table presents recent meta-analyses evaluating the impact of IV iron therapy in heart failure, with a focus on composite outcomes hospitalization reduction, and mortality trends.

CONCLUSION

In recent decades, many studies have contributed to showing the clinical benefit of iron supplementation in patients with chronic diseases and ID. In HF, due to the pathophysiological role of hepcidin, oral administration of iron has not been shown to be as an effective treatment approach. On the contrary, IV iron repletion, without significant side effects, emerged as a proper therapeutic method. Benefits such as improved functional status, reduced HF hospitalization, and cardiovascular mortality justify the cost-effectiveness of IV iron. Notably, meta-analyses have shown a consistent trend toward reducing all-cause mortality, which extends beyond correcting anemia. Hence, IV iron should be viewed primarily as a morbidity-modifying intervention until more conclusive mortality data become available.

In future, new studies should aim for more extended follow-up periods and work towards standardizing IV iron dosing regimens across clinical trials. Well-established study protocols are needed to fully integrate iron repletion into HF management, regardless its phenotype.

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INTRAVENSKA TERAPIJA GVOŽDEM KOD SRČANE ISUFICIJENCIJE SA SMANJENOM EJAKCIONOM FRAKCIJOM: DOKAZI IZ SKORAŠNJIH KLINIČKIH STUDIJA I META-ANALIZA

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Sažetak

Anemija je čest komorbiditet kod pacijenata sa srčanom insuficijencijom (SI) i utiče na njihov morbiditet i mortalitet. Iako je etiologija anemije kod ovih bolesnika višestruka, hronična inflamacija i nedostatak gvožđa najčešći su uzroci. Anemija je snažan prognostički pokazatelj kako u akutnoj, tako i u hroničnoj SI, dok nedostatak gvožđa, čak i u odsustvu same anemije pogoršava simptome, funkcionalni kapacitet i kvalitet života pacijenata. Kod hronične SI često se sreće funkcionalni nedostatak gvožđa, koji nastaje usled poremećaja korišćenja gvožđa, ali može koegzistirati sa apsolutnom deficijencijom, kada same zalihe gvožđa nedostaju.

Terapija intravenskim gvoždem pokazala je brojne koristi u vidu poboljšanja funkcionalnog statusa, ublažava-

nja simptoma i redukcije stope ponovnih hospitalizacija. Na osnovu ovih dokaza, najnovije smernice Evropskog udruženja kardiologa preporučuju suplementaciju gvožđa kod simptomatskih pacijenata sa srčanom slabošću i redukovanom ejakcionom frakcijom (HFrEF). Poslednje meta analize pokazuju trend ka smanjenju ukupne smrtnosti.

Cilj ovog revijalnog članka je da prikaže trenutne uvide u značaj nedostatka gvožđa i terapijske efekte njegove nadoknade kod pacijenata sa HFrEF-om. Pravilno razumevanje ovog stanja i njegovo blagovremeno lečenje predstavljaju važan korak ka individualizovanom i sveobuhvatnom pristupu terapiji SI.

Ključne reči: srčana insuficijencija, intravensko gvožđe, deficijencija gvožđa, anemija

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REVIEW ARTICLE

Targeted drug development in fragile X syndrome: molecular medicine as a key tool in clinical pharmacology

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Summary

The full mutation and epigenetic silencing of the *FMR1* gene lead to a deficiency of its protein, FMRP, resulting in Fragile X Syndrome (FXS). Although significant advances have been made in understanding the molecular mechanisms underlying FXS, no cure or targeted pharmacological treatments have yet been approved for this neurodevelopmental disorder. Current clinical management primarily relies on symptomatic therapies, which often offer limited benefits and do not address the core molecular causes of the condition, especially given the multifaceted roles of the FMRP.

This review highlights the crucial role of molecular insights in guiding the development of drugs for FXS. It provides an overview of existing pharmacotherapies, discusses their benefits and limitations, and emphasizes the unmet need for interventions that target the specific pathways disrupted by *FMR1* dysfunction. Recent and ongoing clinical trials were examined, focusing on how a deeper understanding of FXS molecular biology can inform the design of more effective and precise therapeutic strategies. In summary, key molecular pathways relevant to FXS are presented, and the potential synergy between clinical pharmacology and molecular medicine is discussed as a means to promote the advancement of tailored therapeutic approaches.

Keywords: fragile X syndrome, pharmacotherapy, targeted treatment, *FMR1* gene

INTRODUCTION

Fragile X syndrome (FXS; OMIM: #300624; ORPHA: 908), sometimes referred to as FRAXA syndrome, FraX syndrome, or Martin-Bell syndrome, is a neurodevelopmental condition recognized as the most studied single-gene cause of autism spectrum disorder (ASD), and associated with moderate to severe intellectual disability (ID) in the most affected males with FXS (reviewed in: (1, 2)). According to the Orphanet database, the estimated prevalence of FXS ranges from 1 in 2,400 to 1 in 6,000 in the general population (more information available at www.orpha.net). However, prevalence rates vary across different parts of the world and depend on availability and methods of genetic testing (3). FXS is most commonly caused by a full mutation (FM, >200 CGG repeats) in the *FMR1* gene, which is located at Xq27.3 on the X chromosome, leading to its hypermethylation and transcriptional silencing and the deficit of FMRP, its final product. (4-7). FMRP binds to ribosomes and regulates the translation of specific messenger RNAs (mRNAs) that are essential for synapse formation, which together play a critical role in neurological development and function (4). It is considered an “immediate early protein” at the synapse, coordinating synaptic development, plasticity, and elimination (8). In addition, FMRP is involved in subcellular transport and RNA stability (1, 3, 9). Furthermore, point mutations and deletions within the *FMR1* gene have also been described as causes of FXS (6, 10).

Clinically, FXS is characterized by a combination of neurobehavioral and physical features (reviewed in: (1, 11)). In addition to ID and ASD, individuals with FXS typically exhibit speech and language delays, significant behavioral and neurological challenges, and characteristic physical features. These include a long, narrow face, prominent ears, a high-arched palate, hyperextensible joints, flat feet, and, in males after puberty, macroorchidism (1, 11-14). Symptoms are generally more severe in males, whereas females, due to the presence of two X chromosomes, often present with a milder phenotype (15).

Although the body of knowledge on FXS has grown substantially over the past three decades, no cure or approved targeted treatment exists for FXS. Several clinical trials are ongoing; however, until pharmacotherapies that specifically target the molecular pathways associated with the *FMR1* gene and its product, FMRP, become available, symptomatic treatment remains the foundation of pharmacological management in this population. Building on current scientific understanding, this article aims to provide a comprehensive review of the critical interplay between molecular medicine and clinical pharmacology in advancing drug development for FXS. In other words, this review aims to highlight how a deeper understanding of molecular mechanisms directly supports and informs future drug development efforts, serving as the key tool in clinical pharmacology and targeted drug development.

METHODS

To prepare this review, a comprehensive literature search was conducted using the PubMed, MEDLINE, and Google Scholar databases, as well as the clinical trial registry at clinicaltrials.gov. The search included articles and clinical trials published over the past two decades, with an emphasis on studies published within the last five years. The following keywords and their combinations were used: Fragile X Syndrome (FXS), *FMR1* gene, *FMR1* Protein (FMRP), pharmacotherapy, targeted treatment, and clinical trials. Additional references were identified by manually screening the bibliographies of key articles. Studies were selected based on their relevance to the pathophysiology, molecular targets, and pharmacological treatment approaches for FXS. Specifically, this article reviewed peer-reviewed original studies, reviews, and case reports, without restrictions on inclusion/exclusion criteria, outcome measures, or sample size.

BENEFITS AND LIMITATIONS OF CURRENT SYMPTOMATIC PHARMACOTHERAPY FOR FXS

Available pharmacological therapies give symptomatic benefit and are effective in improving the quality of life in patients with FXS (16). According to previously published data, between 40% and 90% of individuals with FXS, depending on sex and age, have received a prescription for psychotropic medication (17). In 2012, an extensive national caregiver survey in the US included information about 1064 males and 299 females with FXS, where 61% of males and 38% of females were reported to be taking medication for at least one neurological or behavioral symptom (18). The most often treated symptoms were anxiety, with treatment persisting into adulthood. Attention difficulties and hyperactivity were also common symptoms, usually treated throughout adolescence (18). More recent analysis of data from 975 participants from the Fragile X Online Registry with Accessible Research Database (FORWARD) found that 63% of participants used psychotropic medications (17). Most commonly prescribed drugs were (i) selective serotonin reuptake inhibitors (SSRIs) (43%), (ii) stimulants (38%), and (iii) antipsychotics (33%), and these drugs were more frequently used in males, individuals with ASD as a comorbidity in FXS, and adolescents (17). The listed classes of medication are briefly described below.

Sertraline and other SSRIs are very effective in treating anxiety, which affects around 70% to 80% of individuals with FXS (19, 20). SSRIs inhibit the presynaptic reuptake of serotonin, leading to increased synaptic serotonin levels, which are an essential positive mood regulator in the central nervous system (21). Sertraline can be started for anxiety even in young children, and has been shown to have additional positive effects on language and

motor development (19). Although sertraline is usually well tolerated, children may occasionally exhibit signs of behavioral activation, such as restlessness and excitement, particularly with a rapid upward dose titration (22).

Stimulants are first-line therapy for the treatment of attention deficit hyperactivity disorder (ADHD) in children with FXS who are older than 5 years, characterized by a persistent pattern of impulsivity, hyperactivity, and/or inattention (23). Stimulants primarily exert their effects by increasing the levels of dopamine and norepinephrine in the prefrontal cortex, where they have a significant influence on motivation, attention, and impulsivity (24, 25). In individuals with FXS-associated ADHD, stimulants are effective in approximately 70% of cases, with effective doses similar to those in the general population (26). Stimulants are mostly well tolerated, with rare occurrences of serious side effects like palpitations and high blood pressure (27). In FXS, stimulant doses are kept relatively low because higher doses can suppress language, which is a significant side effect to prevent in nonverbal individuals or individuals with low language abilities (23).

Individuals with FXS often exhibit more intense behavioral issues, such as aggression, self-injury, and severe temper outbursts, especially during adolescence (28). These symptoms can be treated with atypical antipsychotics such as risperidone and aripiprazole, which affect serotonergic and dopaminergic receptors in the CNS (26). Antipsychotics are effective, generally safe, and well-tolerated, but must be used with caution due to potential side effects such as weight gain. Indeed, 30–60% of individuals with FXS may have issues with weight gain (29). Aripiprazole may be preferred over risperidone for its lower risk of weight gain (30).

In addition to the described classes of medications, alpha-2 adrenergic agonists, such as clonidine and guanfacine, are also helpful for the treatment of ADHD symptoms, particularly in individuals not responding or not tolerating stimulants, such as children younger than 5 years (2). These agents activate presynaptic alpha-2 adrenergic receptors, leading to increased norepinephrine levels in the prefrontal cortex, which improves attention modulation (31). Clonidine may be helpful for children with FXS who also have sleep issues (32). Guanfacine is recommended for treating problematic behavior during the day because it causes less drowsiness than clonidine (33). Drowsiness may occur as a side effect after initiating therapy with either drug, particularly with a rapid upward dose titration. In addition, to reduce the risk of rebound hypertension in clonidine use, abrupt withdrawal should be avoided (23).

Melatonin is the primary treatment used to manage sleep problems in FXS, which affects between 27% and 77% individuals and are usually mild-to-moderate (12). Clonidine and guanfacine are other possible options if melatonin is ineffective (2, 23).

Based on previous pharmacological studies, it is evident that more than two-thirds of individuals with FXS have required pharmacological treatment at some point in their lives (17). This highlights the necessity and importance of using pharmacotherapy in the treatment of FXS and its associated symptoms.

Nevertheless, currently available treatments are not specific and only partially alleviate symptoms. Therapeutic effects are variable, and many of the drugs have side effects that can complicate compliance and decrease therapeutic benefit (23). Moreover, complex underlying molecular mechanisms in FXS often require the administration of multiple drugs, leading to a high prevalence of polypharmacy (34).

Although symptomatic pharmacotherapy remains the mainstay of current clinical practice, it fails to treat the root cause of FXS (23). A significant limitation of symptomatic treatment is that it targets downstream behavioral manifestations without correcting the underlying molecular pathology caused by the absence of functional FMRP. As a result, this has led to growing interest in the development of targeted therapies aimed at reversing dysregulated pathways, such as metabotropic glutamate receptor 5 (mGluR5) signaling, gamma-aminobutyric acid (GABA) deficiencies, mammalian Target of Rapamycin (mTOR) activation, and endocannabinoid dysfunction, as discussed in the next section of this review.

MOLECULAR PATHWAYS RELEVANT TO DRUG DEVELOPMENT IN FXS

Numerous studies have demonstrated that low levels or loss of FMRP disrupts brain protein synthesis by changing neural mRNAs, resulting in reduced quantity and integrity of neuronal dendrites and dendritic spines (8, 9, 35, 36). FMRP inactivation may also lead to an imbalance between neuronal excitation and inhibition. The activation of mGluR5 in the absence of FMRP in brain cells promotes the expression of glutamate receptors (37, 38). Enhanced glutamatergic signaling via mGluR5 leads to increased protein synthesis and defects in synaptic plasticity, contributing to cognitive dysfunction and behavioral abnormalities (38–41). In addition to altered glutamatergic signaling, the lack of FMRP has been demonstrated to reduce the synthesis of both GABA and its receptor (40, 42). As a result, an imbalance in these neurotransmitters may lead to disturbances in neuronal plasticity (43). Moreover, dysfunction of the endocannabinoid system has been implicated in FXS pathology (44, 45).

FMRP is also associated with ion channel control and is essential for the functions necessary for effective synaptic transmission (46). FMRP regulates action potentials through the large conductance Ca^{2+} -activated potassium BK channel by interacting with the sodium-activated potassium Slack channels (47–49). In addition, FMRP

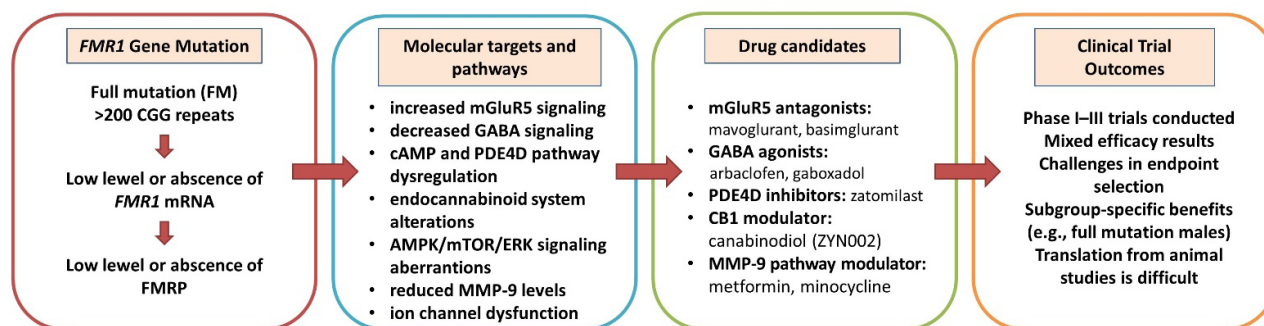


Figure 1. From gene mutation to clinical trials: molecular pathways and targeted therapies in fragile X syndrome

Abbreviations: *FMR1* gene - fragile X messenger ribonucleoprotein 1 gene; FMRP - fragile X messenger ribonucleoprotein; mGluR5 - metabotropic glutamate receptor 5; GABA - gamma aminobutyric acid; cAMP - cyclic adenosine monophosphate; PDE4D - phosphodiesterase 4D; CB1 - cannabinoid receptor type 1; AMPK - AMP-activated protein kinase; mTOR - mechanistic target of rapamycin; ERK - extracellular signal-regulated kinase; MMP-9 - matrix metalloproteinase 9.

regulates the voltage-gated potassium channels Kv3.1b and Kv4.2 (49). In the absence of FMRP, weak synaptic connections cannot undergo sufficient brain plasticity, thereby hindering normal intellectual growth (46).

FMRP also interacts with a large number of mRNA targets that encode proteins involved in synaptic structure and function, such as matrix metalloproteinase 9 (MMP-9) (50-52) and postsynaptic density protein 95 (PSD-95) (53). Finally, modest levels of FMRP were identified in the cell nucleus, indicating that FMRP may have many other previously undiscovered roles, such as DNA expression through DNA stabilization and epigenetic regulation, and DNA damage responses (54, 55).

The increasing understanding of these molecular mechanisms has not only improved our knowledge of the pathophysiology of FXS but also created a compelling framework for the development of targeted therapies in FXS (11). This involves identifying therapeutic targets such as matrix metalloproteinase 9 (MMP-9), mGluR5, GABA receptors, the PI3K-AKT-mTOR and ERK-MAPK signaling cascades, and components of the cAMP pathway (2, 23, 56, 57). Preclinical studies using *Fmr1* knockout mice, zebrafish, and *Drosophila* models have been crucial for identifying and validating therapeutic targets and testing pharmacological agents (58). By targeting the fundamental synaptic and molecular abnormalities of FXS, several promising drugs, including metformin, minocycline, cannabidiol (CBD), and other mGluR5 antagonists, have shown therapeutic promise (23, 56, 57).

While it has been difficult to successfully translate these findings into human trials, due to the variable clinical presentation of FXS, difficulties in selecting appropriate outcome measures, and the need for molecularly stratified trial populations (59, 60), these challenges have prompted the development of precision medicine approaches. Namely, medical interventions are tailored to individual genetic and molecular characteristics, including methylation status and FMRP expression levels (61, 62). Incorporating molecular biomarkers into clinical trial designs has begun to improve therapeutic endpoints

and participant selection, ultimately increasing the likelihood of treatment success (61, 63).

As a result, FXS has evolved from a clinically defined disorder to a molecularly characterized condition that exemplifies the promise of mechanism-based therapeutics. As such, it offers a powerful model for translational research and the application of molecular medicine in clinical pharmacology (37).

The following paragraph of this review article critically examines the most important clinical trials and drug development in FXS, based on its molecular landscape and the implications for targeted therapies, incorporating both established knowledge and recent advances. **Figure 1** provides a schematic illustration of the relationship between molecular medicine and clinical pharmacology in the field of FXS.

TARGETED DRUG DEVELOPMENT IN FXS

Metabotropic glutamate receptor 5 (mGluR5) pathway in FXS and related drug development

As mentioned above, one of the most studied molecular pathways in FXS is the mGluR5 signaling pathway. The absence of FMRP in FXS is believed to cause excessive activation of group 1 metabotropic glutamate receptors (mGluR1 and mGluR5), as proposed by the mGluR theory (64). This results in exaggerated long-term depression (LTD) and impaired neural signaling in parts of the brain like the hippocampus, which leads to the cognitive and behavioral symptoms seen in FXS (64).

Promising results from preclinical studies led to human clinical trials with mGluR5 antagonists, aimed at investigating their efficacy, safety, and tolerability. Mavoglurant (AFQ056) was identified as a non-competitive mGluR5 antagonist (65) and was further developed and entered human trials. A small, randomized, double-blind, crossover study of 30 adult males with FXS showed promising results: AFQ056 significantly improved behavior, as

measured by the Aberrant Behavior Checklist-Community for FXS (ABC-C_{FX}), in participants with complete *FMR1* promoter methylation and no detectable *FMR1* mRNA (66). However, the two Phase 2b, double-blind, placebo-controlled trials with mavoglurant failed to reach the primary efficacy endpoint of behavioral improvement, as measured by the ABC-C_{FX}. Studies included 139 adolescents (aged 12 to 17 years) and 175 adults (aged 18 to 45 years) with FXS, who were randomized to receive either a placebo or mavoglurant (25, 50, or 100 mg twice daily) for 12 weeks, after being stratified based on their methylation status (67). Similar disappointing results were obtained with basimglurant, a mGluR5 negative allosteric modulator (NAM). Two Phase II clinical trials, in children aged 5–13 years and in adults and adolescents aged 14–50 years, examined the efficacy of basimglurant based on changes from baseline scores in behavioral symptoms, using the Anxiety Depression and Mood Scale (ADAMS) total score as the primary efficacy endpoint (68). Two dosages of basimglurant (0.5 mg and 1.5 mg) were tested in participants over a 12-week treatment period in these randomized, double-blind, placebo-controlled, parallel-design trials (11, 68). Previous studies with mGluR5 antagonists have pointed to the need for future FXS trials to optimize endpoints, target younger age groups, extend study duration, reduce patient variability, and better control for placebo effects using biomarkers (2).

More recently, FXLEARN, a double-blind, placebo-controlled, parallel-group, flexible-dose, forced-titration design study with AFQ056 (mavoglurant), was conducted. A large multisite trial involving children aged 3–6 years with FXS found that the mGluR5 inhibitor AFQ056 did not show a significant improvement in language abilities compared to the placebo (69). Different publications analyzed blood biomarkers obtained from samples from FXLEARN study participants, including *FMR1* genotyping, methylation, mRNA, MMP-9, and Akt/mTOR pathway markers (70). Also, it found no treatment-related changes, supporting the lack of clinical benefit observed (69, 70).

GABA signaling pathway in FXS and related drug development

Dysregulation of the inhibitory GABAergic system is also involved in the pathophysiology of FXS. An imbalance in neuronal inhibition can contribute to anxiety, sensory hypersensitivity, and seizures, which are frequently present in FXS (2). The absence of FMRP leads to decreased expression of GABA_A receptor subunits, as well as enzymes associated with GABA synthesis and metabolism, including Abat, Gad1, and Gad2 (42, 64).

Arbaclofen, a selective GABA_B agonist, was tested in two Phase 3, placebo-controlled, flexible-dose trials; one in adolescents and adults (aged 12–50 years) and one in children (aged 5–11 years). The primary efficacy

endpoint was assessed by using the social avoidance subscale of the ABC-C_{FX}. Arbaclofen failed to meet primary endpoints in both phase 3 trials. Still, in the child study, the group receiving the highest dose (10 mg twice daily) showed significant improvement compared to placebo on the ABC-C_{FX} Irritability subscale and Parenting Stress Index (71). In both studies, side effects were primarily mild, and overall, arbaclofen was well tolerated (71).

Another drug, ganaxolone, a positive allosteric modulator (PAM) of GABA_A receptors, was also tested in a Phase 2 randomized, double-blind, placebo-controlled, crossover trial involving 59 children with FXS, aged 6–17 years. Ganaxolone demonstrated a favorable safety profile; however, there was no statistically significant difference in the primary endpoint, CGI-I, between the ganaxolone and placebo groups. Nevertheless, post hoc analyses showed ganaxolone efficacy in subgroups of participants with higher baseline anxiety and in those with low cognitive abilities (72).

Gaboxadol (OV101), a δ -subunit-selective, extra-synaptic GABA_A receptor agonist, was evaluated for safety, tolerability, efficacy, and optimal dosage regimen in a phase 2a randomized, double-blind, parallel-group clinical study, known as the ROCKET study. The results showed that gaboxadol was generally well tolerated, with no serious adverse events reported. Based on CGI-I scores, approximately 60% of 23 participants were identified as treatment responders (73). These findings support the continued investigation of gaboxadol in larger, placebo-controlled trials, and one such trial is currently ongoing.

Phosphodiesterase-4D pathways in FXS and related drug development

A deficiency of FMRP also leads to dysregulation of cyclic adenosine monophosphate (cAMP) signaling, characterized by reduced levels of cAMP in FXS. cAMP is a crucial molecule regulating synaptic function, and its degradation is mediated by phosphodiesterases (PDEs) (74). Inhibiting PDEs represents a therapeutic strategy aimed at restoring normal cAMP signaling, and PDE inhibitors have shown positive effects on behaviors in animal models of FXS (11).

BPN14770 (zatolmilast), a selective PDE4D inhibitor, was evaluated for safety, tolerability, and cognitive efficacy in a phase 2 randomized, placebo-controlled, two-way crossover trial in 30 adult males aged 18–41 years with FXS (75). The study met its primary endpoint, demonstrating that BPN14770 (zatolmilast) was well-tolerated with no significant differences in adverse events between the treatment and placebo arms (75). Significant improvements were observed in secondary efficacy outcomes, including cognition and daily functioning. Notably, participants demonstrated cognitive benefits on the National Institutes of Health (NIH) Toolbox assess-

ments, specifically in Oral Reading Recognition, Picture Vocabulary, and the Crystallized Cognition Composite scores. Caregiver-reported visual analog scales also indicated clinically meaningful improvements in language and daily functioning (75). This study opened an exciting avenue for future research on this compound, as it is the first clinical trial to demonstrate cognitive improvements in individuals with FXS (2). The EXPERIENCE (Evaluation of Fragile X Experience in Cognition Expression) Clinical Trials, a Phase 2b/3 clinical trial, is currently underway. It consists of two randomized, double-blind, placebo-controlled trials: in adolescent males aged 9-17 years and in adult males aged 18-45 years. The main objective of these studies is to assess the cognitive effects of zatolmilast, using the Crystallized Cognition Composite Score from the NIH Toolbox Cognitive Battery (NIH-TCB) as the primary measure. Secondary endpoints include evaluations of daily functioning, language, emotional/behavioral, and other cognitive domains assessed by the NIH-TCB. Additionally, studies evaluating the safety and tolerability of the drug are conducted.

Cannabinoid pathway in FXS and related drug development

The absence of FMRP leads to alterations in the endocannabinoid system, particularly to reduced levels of endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), as well as desensitization of cannabinoid receptor 1 (CB1). Cannabidiol (CBD), a non-psychoactive compound from cannabis, has emerged as a potential therapy and is commonly used by families to manage anxiety, sleep problems, and tantrums in children with FXS. CBD is a negative allosteric modulator of CB1, which helps restore synaptic balance.

Additionally, CBD can bind to multiple receptors, including those involved in serotonin, dopamine, and GABA signaling, which can repair the imbalance between excitatory and inhibitory signaling observed in FXS (76). ZYN002, a transdermal CBD gel, is the most studied CBD-based treatment in clinical trials for FXS, offering systemic delivery without psychoactive effects of THC. A phase 1/2, open-label, multi-site trial of transdermal CBD (ZYN002) assessed its safety, tolerability, and efficacy in 20 children and adolescents, aged 6-17 years with FXS (77). Treatment with ZYN002 was associated with good tolerability, with only minor adverse effects observed. The study met the primary efficacy endpoint, as measured by a statistically significant reduction in the ADAMS total score. Additionally, the trial achieved its secondary endpoints, demonstrating significant improvements in behavioral symptoms, anxiety, and quality of life (77). These findings led to the CONNECT-FX study, a Phase 3 randomized, double-blind, placebo-controlled trial that included 212 children and adolescents aged 6-17 years with FXS (76). In this 12-week trial, par-

ticipants received 250 mg or 500 mg of ZYN002 twice daily or a placebo. The primary endpoint, improved social avoidance on ABC-C_{FX}, was not met by the whole cohort. However, a post hoc analysis indicated that participants with *FMR1* gene promoter methylation of 90% or higher experienced significant benefits. In this subgroup, ZYN002 also led to improvement in caregiver-rated social avoidance, social interaction, and irritable behaviors. Consistent with observations from the earlier open-label trial, ZYN002 was found to be safe and well-tolerated (76). Based on learnings from the CONNECT-FX trial, another trial, RECONNECT, is ongoing and is expected to provide further data on the effectiveness of ZYN002. RECONNECT is a phase 3, multi-center, randomized, double-blind trial in children, adolescents, and young adults, aged 3-30 years, with FXS. The RECONNECT trial improves upon CONNECT-FX by focusing on patients with fully methylated *FMR1* genes, expanding to three weight-based dosing levels (250, 500, and 750 mg/day), and extending treatment to 18 weeks. It also utilizes an FXS-anchored Clinical Global Impression of Severity/Change (CGI-S/I) scale, which is anchored on three behavioral symptoms: social avoidance, social interactions, and irritability. The CANAX study is an upcoming randomized, double-blind, placebo-controlled, single-center, crossover trial investigating the effects of an oral CBD solution on anxiety and GABAergic function in individuals with FXS. The study will evaluate the effects of oral CBD on anxiety, disruptive behavior, and behavioral inhibition.

Matrix metalloproteinase-9 (MMP9) molecules as a target in drug development in FXS

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent enzyme involved in remodeling the extracellular matrix, and it is essential for brain development and synaptic plasticity (78). Normally, FMRP binds to MMP-9 mRNA in dendrites and inhibits its translation (79). The absence of FMRP in FXS leads to excessive local synthesis and activity of MMP-9 at synapses, which causes aberrant synaptic architecture and cognitive impairments (79). Measurement of MMP-9 levels has shown significantly higher active MMP-9 plasma levels in FXS individuals compared to healthy controls (50). In individuals with FXS, a high active MMP-9 level is correlated with aberrant behavior (measured using the ABC-C_{FX}) and an ADAMS score, suggesting its role as a biomarker and therapeutic target (50).

Minocycline, a tetracycline antibiotic known to inhibit MMP-9 activity, has been tested in clinical trials for FXS. An open-label add-on treatment trial has shown significant improvement in ABC-C Irritability Subscale and CGI-I scores in 20 FXS participants treated with minocycline (80). A randomized, double-blind, placebo-controlled, crossover trial further supported these findings.

	Symptomatic Therapy	Targeted Therapy
Drug examples:	Methylphenidate Aripiprazole Risperidone Melatonin	Mavoglurant (mGluR5 antagonists) Arbaclofen (GABA _B agonist) Metformin (MMP-9 pathway) Gaboxadol (GABA _A agonist)
Mechanism:	Non-specific action on neurotransmitters	Direct modulation of FMRP-regulated pathways
Evidence:	Widely used off-label Some efficacy for behavioral symptoms	Mostly in clinical trials Mixed success in trials, better results in subsets
Limitations:	Does not address core pathophysiology Side effects Trial-and-error approach	Translational gap from animal models Need for biomarkers and stratification Lack of efficacy (discontinued trials)
Clinical use:	Standard clinical practice	Investigational or compassionate use
Goal:	To alleviate observable symptoms: anxiety, ADHD, irritability, sleep issues	To reverse underlying molecular dysfunction: mGluR5 signaling, GABAergic tone, protein synthesis control

Figure 2. An overview of symptomatic and targeted treatment in fragile X syndrome

Abbreviations: ADHD - attention deficit hyperactivity disorder; mGluR5 - metabotropic glutamate receptor 5; GABA - gamma aminobutyric acid; GABA_A - gamma aminobutyric acid type A receptor; GABA_B - gamma aminobutyric acid type B receptor; MMP-9 - matrix metalloproteinase 9.

The trial included 66 participants with FXS, aged 3.5 years to 16 years, confirming statistically significant improvement in CGI-I scores, along with greater improvement in anxiety and mood-related behaviors, in the minocycline group compared to the placebo (81).

Metformin, an antidiabetic drug, reduces MMP-9 levels by activating both AMP-activated protein kinase (AMPK)-dependent and independent pathways, leading to suppression of the aberrant mTORC1 signaling observed in FXS (82, 83). The first published case series described improvement in behavior and language in seven individuals with FXS treated with metformin (84). No significant side effects were observed, and metformin treatment led to weight loss as seen in three cases with obesity (84). Another case series of nine children aged 2-7 years with FXS, reported that clinical treatment with metformin improved behavior and language, as measured by improvement in ABC-C_{FX} and Mullen Scales of Early Learning (MSEL) before and after metformin treatment (85). The authors of the publication emphasized the need for a controlled trial of metformin in children younger than 7 years, since their brains are still developing and may benefit more from the medication (85).

Contrary to typical IQ decline seen in FXS, clinical treatment with metformin in two adult men with FXS for one year showed significant cognitive improvement,

measured as increases in Full Scale IQ, Nonverbal IQ, and Verbal IQ on the Stanford-Binet Intelligence Scale, Fifth Edition (SB-5)(86). Additionally, significant improvements in communication, social engagement, and behavior, along with better eating habits, were observed in both individuals (86). Protic et al (2019) reported the first case of a 14-year-old pubertal male with FXS, who did not develop macroorchidism after two years of treatment with metformin (87).

An open-label, phase 2 study in 15 individuals with FXS (aged 17-44 years) who received metformin 500 mg twice daily for nine weeks showed a favorable safety profile of metformin in normoglycemic individuals (88). Using transcranial magnetic stimulation to assess excitatory and inhibitory neural mechanisms, the study revealed elevated corticospinal inhibition mediated by GABAergic pathways (88). Recently, a multi-site, double-blind, placebo-controlled trial has been conducted, but the results have not yet been published and are highly anticipated (58). This 4-month trial included individuals aged 6-25 years with FXS. It was designed to evaluate the effects of metformin on expressive language, as well as its potential benefits on challenging behaviors, cognition, eating behavior, adaptive functioning, mood and anxiety symptoms, sleep habits, ADHD symptoms, and overall quality of life. A longitudinal follow-up study, published

in 2024, included individuals with FXS who completed the previously described 4-month clinical trial and were followed up for a period of 1 to 3 years (89). Twenty-six participants with FXS, aged 6–25 years, were recruited from three different sites and treated with metformin at doses ranging from 500 to 1,000 mg twice daily. Baseline and follow-up assessments conducted after at least one year of metformin treatment revealed no significant changes over time in nonverbal IQ and adaptive behavior, as measured by the Leiter-III and Vineland-III, respectively. More importantly, the results indicated stability in cognition and adaptive behavior, suggesting that metformin may help prevent the typical decline seen in FXS. However, the small sample size and short follow-up limited the conclusions (89). Currently, two additional clinical trials are recruiting individuals with FXS: one in Canada for participants aged 6 to 35 years, and another in China for participants aged 2 to 16 years (58). Metformin shows excellent promise as a targeted treatment for FXS; however, controlled trial results are needed to confirm its effectiveness.

An overview of symptomatic and targeted treatment in FXS is presented in [Figure 2](#).

CONCLUSION

FXS remains without a cure or an approved targeted pharmacological therapy despite substantial progress in understanding its molecular basis. Current treatments are primarily symptomatic and do not address the underlying mechanisms driven by *FMR1* dysfunction. Advances in molecular medicine are paving the way for more rational and precise drug development approaches in FXS. Integrating clinical pharmacology with molecular insights is essential for translating these discoveries into effective therapies. Continued research into key signaling pathways will be crucial for achieving personalized and disease-modifying treatments for individuals with FXS.

In addition, based on the previous results of failed and successful clinical trials in the field of fragile X, there is a need to reassess outcome measures and revise recommendations for FXS, as presented in the review article published by Budimirovic et al. in 2017 (90). Clinical trials in FXS often failed because appropriate outcome measures had not been fully developed. It appeared that the ultimate success of clinical trials in FXS largely depended on the choice of outcome measures; some trials were judged as unsuccessful not because the treatments were ineffective, but because the selected endpoints were not optimally aligned with the specific challenges of this field. Although progress had been made in creating cognitive and behavioral instruments, most tools remained only of moderate quality, with limited evidence of reliability, validity, and sensitivity to treatment effects. Biomarkers and other objective measures, which could have provided

more quantitative endpoints, had advanced slowly, partly due to the industry's reluctance to invest in costly projects with uncertain regulatory approval. As a result, trials relied heavily on parent-reported outcomes in the behavioral domain, which lacked objectivity and consistency. Despite continuous efforts and some tangible progress, the limited availability of validated, clinically meaningful endpoints hindered the demonstration of treatment efficacy and contributed to the failure of past studies.

From a clinical perspective, certain drugs already available on the market (e.g., metformin, sertraline, etc.) and their combinations entered clinical use as off-label therapies for FXS even before results from clinical trials were available. This approach was supported by the clinical experience of experts in the field and the positive effects observed in individual cases. However, once the results of the ongoing clinical trials in FXS are published, we will have much more data to guide our decisions in selecting pharmacotherapy for individuals diagnosed with FXS.

Currently, there are no approved medications that target the root cause of FXS; available pharmacological strategies only address comorbid behavioral and psychiatric symptoms. As summarized in the paper published by Protic and Hagerman in 2023 (23). Gene-based approaches, including antisense oligonucleotide (ASO) therapy, adeno-associated virus (AAV) vectors, and CRISPR-based techniques, are being explored to reactivate or correct the *FMR1* gene. Still, none have yet advanced to clinical trials in FXS. Significant obstacles remain, particularly the delivery of therapeutic agents across the blood–brain barrier, the risk of uneven distribution within brain tissue, vector-related toxicity, and the need for sustained, regulated expression without off-target effects. Advances in nanotechnology, viral vector engineering, and delivery methods are being investigated to overcome these limitations. Importantly, the timing of FMRP restoration is critical, as earlier interventions—ideally before or during the onset of symptoms—may offer greater therapeutic benefit, although this raises complex ethical considerations. Long-term or even lifelong administration may also be necessary, underscoring the need for careful evaluation of safety, efficacy, and feasibility in future gene therapy trials for FXS.

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List of all abbreviations:

2-AG - 2-arachidonoylglycerol

ABC-C_{FX} - Aberrant Behavior Checklist-Community for FXS

ADAMS - Anxiety Depression and Mood Scale

ADHD - attention deficit hyperactivity disorder

AEA - anandamide

AMPK - AMP-activated protein kinase

ASD - autism spectrum disorder

cAMP - cyclic adenosine monophosphate

CB1 - cannabinoid receptor 1

CBD - cannabidiol

FM-full mutation

FMR1 gene - Fragile X Messenger Ribonucleoprotein 1 gene

FMRP - FMR1 protein

FXS - fragile X syndrome

GABA - gamma-aminobutyric acid

ID - intellectual disability

mGluR5 - metabotropic glutamate receptor 5

MMP-9 - matrix metalloproteinase 9

mRNAs - messenger RNAs

MSEL - Mullen Scales of Early Learning

NAM - negative allosteric modulator

PAM - positive allosteric modulator

PDEs - phosphodiesterases

PSD-95 - postsynaptic density protein 95

SSRIs - selective serotonin reuptake inhibitors

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RAZVOJ CILJANE FARMAKOTERAPIJE ZA FRAGILNI X SINDROM: MOLEKULARNA MEDICINA KAO KLJUČNO SREDSTVO U KLINIČKOJ FARMAKOLOGIJI

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Sažetak

Puna mutacija i epigenetsko utišavanje gena *FMR1* dovode do nedostatka njegovog proteina, FMRP, što rezultira fragilnim X sindromom (FXS). Iako je ostvaren značajan napredak u razumevanju molekularnih mehanizama koje su u osnovi FXS-a, za ovaj neurorazvojni poremećaj još uvek ne postoji lek niti je odobrena ciljana farmakološka terapija. Trenutno lečenje se uglavnom oslanja na simptomatsku terapiju, koja često donosi ograničene koristi i ne utiče na osnovne molekularne uzroke poremećaja.

Ovaj pregledni rad ukazuje na značaj integracije molekularnih saznanja u procesu razvoja lekova za FXS. Prikazan

je pregled postojeće farmakoterapije, sa analizom njihovih prednosti i ograničenja, uz naglašavanje potrebe za terapijama koje ciljaju specifične puteve poremećene disfunkcijom *FMR1* gena. Razmatrana su savremena i tekuća klinička ispitivanja, sa fokusom na to kako dublje razumevanje molekularne biologije FXS-a može doprineti razvoju efikasnijih i preciznijih terapijskih strategija. Na kraju, analizirani su ključni signalni putevi uključeni u patofiziologiju FXS-a i diskutovano je na koji način klinička farmakologija i molekularna medicina mogu zajednički doprineti razvoju personalizovanih terapija za osobe sa ovim složenim poremećajem.

Ključne reči: fragilni X sindrom, farmakoterapija, ciljana terapija, *FMR1* gen

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REVIEW ARTICLE

The nine most common misconceptions in the diagnosis and treatment of glaucoma

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Summary

Diagnosis and treatment of glaucoma have advanced significantly in the past 20 years, but there are still some misconceptions about this group of diseases in everyday ophthalmological practice. The goal of this review is to point out the most common misconceptions in the diagnosis and treatment of glaucoma, which should improve the quality of treatment for our patients.

A review of research articles with the keywords “glaucoma,” “misconception,” and “intraocular pressure measurement,” published in the PubMed database as of June 30, 2025, was conducted.

Some of the most common misconceptions in the diagnosis and treatment of glaucoma are related to the method of measuring intraocular pressure, the time when the measurement is performed, subtracting/adding values according to the nomogram for pachymetry, banning caffeine intake for glaucoma patients, and recommendations for physical activity in all forms of glaucoma.

While significant advancements have been made in the diagnosis and treatment of glaucoma, persistent misconceptions among ophthalmologists must be addressed to improve patient outcomes and quality of life.

Keywords: glaucoma, diagnosis, treatment, misconceptions

INTRODUCTION

Glaucoma is a group of chronic, progressive, neurodegenerative diseases most often, but not exclusively, associated with elevated intraocular pressure (IOP), and also one of the leading causes of blindness in the world. Current estimates indicate that approximately 80 million people are affected by this incurable disease (1), with the number projected to rise to 111 million by 2040.

While glaucoma cannot be prevented or cured, it can be managed effectively through awareness of risk factors, early diagnosis, and adequate treatment (2). Over the past two decades, significant advancements have been made in the diagnosis and treatment of glaucoma. However, several misconceptions about this group of diseases persist. Given that not all ophthalmologists, particularly general practitioners, specialize in glaucoma management, it is essential to address and correct these common misunderstandings.

The purpose of this review is to highlight the most common misconceptions in the diagnosis and treatment of glaucoma so as to improve clinical practice. This paper draws on a comprehensive literature review, with the selection of misconceptions informed by thirty years of experience in glaucoma practice. The misconceptions outlined below are the ones most frequently encountered over that period.

A review of research articles was conducted using the keywords “glaucoma”, “misconception”, “intraocular pressure measurement and phasing”, “coffee and tea consumption”, “pachymetry nomogram”, and “physical activity”. The search included studies published in the PubMed database as of June 30, 2025. To ensure accuracy, searches were performed separately for each category. The total number of research papers found was over 3400 (glaucoma/misconception 24, glaucoma/intraocular pressure measurement 2761, glaucoma/phasing 51, glaucoma/pachymetry nomogram 2, glaucoma/caffeine consumption 72, glaucoma/physical activity 491).

The following are the most common misconceptions encountered in daily glaucoma practice:

Misconception number 1 - Those who do not have high intraocular pressure (IOP) do not have glaucoma

Normal IOP generally falls within the range of 10–21 mmHg (3, 4). Over the course of my 30 years in ophthalmology practice, I have noticed a tendency to overlook comprehensive IOP measurements when the value hovers around 16 mmHg. This seemingly “magic” number often fosters complacency, prompting some practitioners to perform only a single, superficial measurement. Studies conducted on large populations in the United States indicate that the average IOP is between 15 and 16 mmHg, and approximately 95% of people have an IOP between

10 and 21 mmHg (5). We do not actually know what percentage of the normal population has an IOP of 16mmHg.

On the other hand, there is an important issue of normal tension glaucoma (NTG), which represents the cases of glaucomatous optic neuropathy with IOP within normal limits. NTG is present in 10% to 48% of all primary open-angle glaucoma (POAG) patients in the US, Europe, and Scandinavia, and even in 66% of patients in Japan (6). Since IOP is the only modifiable factor in glaucoma treatment, it is critical to measure it accurately and consistently. Quite often, glaucoma is not a disease that imposes itself with its signs and symptoms, but rather one that requires us to “actively look for”. The first step is to check the IOP multiple times (1) precisely. It is strongly recommended that glaucomatologists perform their own IOP measurements for every patient, rather than relying on previous reports, to avoid missing potential glaucoma cases.

Misconception number 2 - Non-contact tonometers are better than Goldmann applanation tonometry (GAT)

The gold standard for IOP measurement was and remains Goldmann’s applanation tonometer (GAT) (7). Moreover, it is recommended always to measure IOP using the same tonometer (in the same way) (8). Most ophthalmologists worldwide rely on GAT and, in some cases, the Icare tonometer.

One of the main points emphasized by advocates of non-contact tonometry is the speed with which measurement results are obtained, the lack of contact between the tonometer and the eye, the need to use a topical anesthetic, and the potential risk of contamination. In the era of COVID-19 and the risk of transmission of other infectious diseases, the methods for disinfecting and sterilizing contact tonometer prisms are clearly defined, and disposable prisms are also available for GAT (9). Non-contact tonometers tend to overestimate IOP, are not widely used in glaucoma clinics (10-12), but are very popular among optometrists, where they are offered as a quick and convenient way to measure IOP. We must be aware that an ophthalmologist can only recommend the use of a non-contact tonometer in cases of population screening.

When discussing the reliability of the obtained IOP values, we must also mention the calibration of the tonometer, as one of the most common misconceptions around Goldmann applanation is the “set it and forget it” practice. Calibration is crucial for reliable glaucoma diagnosis and management, as inaccurate IOP readings can lead to misdiagnosis or inappropriate treatment. The calibration process itself is not complicated and does not take long, and the instructions are provided with the tonometer. In extensive ophthalmology facilities, there is regular technical maintenance of the equipment. However, in smaller facilities, it’s worth reminding ophthalmologists that they can either learn this technique themselves or request

regular calibration of the tonometers. The recommendations for regular GAT calibration are once a month (13).

Misconception number 3 - The time of day when IOP is measured does not matter

IOP is not a static value; it fluctuates throughout the day, with peak levels typically occurring between 6 and 8 AM. Since mean IOP is a strong predictor of glaucomatous damage, understanding its fluctuations over a 12- or 24-hour period is critical for effective treatment (14).

Thus, it is necessary to measure IOP at different times of the day, both in glaucoma patients and in glaucoma suspects (15, 16).

Recording the time when the IOP was measured by GAT (17) is advised not only for making a correct glaucoma diagnosis, but also for the purpose of evaluating the effectiveness of antiglaucoma drugs, and achieving better glaucoma treatment outcomes (18, 19).

Interestingly, IOP has been found to vary by season and day of the week, being higher in winter than in summer and lower on Fridays compared to other days (20, 21).

Misconception number 4 – IOP phasing is only valid before starting treatment

IOP phasing, which involves monitoring IOP at regular intervals during the day or over 24 hours, is traditionally performed before establishing a glaucoma diagnosis. However, it remains valuable even for patients undergoing treatment, as it provides critical data on the effectiveness of medication. Continuous IOP monitoring devices, such as contact lenses with IOP sensors, can offer detailed reports on IOP fluctuations (22, 23). When doing it by GAT, it is usually done every 2 hours during ophthalmological office hours. Devices for continuous IOP measurement give us a detailed report on specific values and times when the IOP reading was taken, similar to a blood pressure holter. However, in daily outpatient work, we most often use GAT during day hours (7-21h), which provides us with valuable additional information in the management of glaucoma patients (24).

There is a reasonable reason to do IOP phasing during treatment as well, when IOP level is checked during day hours along with regular therapy, as a way to evaluate the effectiveness of glaucoma medication therapy. A single measurement of IOP can be misleading if the values are within normal limits. It is advisable to do this extra-prolonged IOP checking in cases where there is glaucoma progression despite reaching IOP target values (25-28).

Misconception number 5 - IOP values must always be adjusted using CCT nomograms

Numerous publications over the past 50 years have shown that central corneal thickness (CCT) has a significant

impact on IOP measurement and is also of great importance for glaucoma progression. According to the European Glaucoma Society Guidelines, a thin central cornea is a considerable risk factor for the onset and progression of glaucoma, but is no longer considered an independent risk factor (29-31).

CCT influences the accuracy of most tonometers, even the most precise ones, such as GAT. Thinner CCT is associated with a higher risk of conversion of ocular hypertension (OH) to glaucoma, and a higher risk of glaucoma progression. To better understand the relationship between CCT and IOP, and to estimate the actual level of IOP more accurately, several tables of nomograms were created. However, those conversion tables are not recommended, since they are not validated, and the relationship between measured IOP and CCT values is not linear. As an unwritten rule, it is understood that for every 50-micron increase in CCT, the IOP should be lowered by 2.5 mmHg (30). In daily clinical practice, the primary concern is whether the cornea is thick or thin. The advice is not to recalculate the IOP values based on CCT; instead, what matters are the actual IOP values measured at that specific moment (32, 33).

Misconception number 6 – In glaucoma follow-up, visual field, OCT, and HRT can be done on different devices.

All glaucoma associations recommend that diagnostic procedures should always be performed using the same devices and the same software, so that the findings can be compared and a conclusion about possible progression can be reached. For visual field testing, the same device and the same strategy are recommended for follow-up tests, and for OCT disc/retinal nerve fiber layer/macula, using the same instrument with software-based analysis. It is strongly recommended that the patient we monitor be always tested on the same machine and with the same program (34, 35). Because different perimeters can have different lighting parameters from the stimulus, and the backlight values in decibels can differ significantly from each other even if they are of the same type, it is not advised to compare visual field test results done on different devices. Standardized visual field testing conditions ensure accurate and reliable results by controlling key variables. These conditions involve patient positioning, refractive correction, fixation, and stimulus presentation, all aimed at minimizing errors and maximizing the detection of visual field defects. This is also the reason why the perimeter needs to be located in a room with controlled conditions, especially in terms of lighting.

Regarding imaging devices, variability in these devices can lead to inconsistent results and hinder the ability to track disease progression (34).

Misconception number 7 - People with glaucoma should avoid coffee and tea

Most medical doctors, including ophthalmologists, will advise a person with glaucoma not to drink coffee or tea/tea or to limit their consumption, and to drink decaffeinated coffee instead. The belief that caffeine consumption worsens glaucoma is mainly anecdotal.

Numerous studies have been conducted, and numerous results have been published on this topic. A meta-analysis showed that coffee consumption does not affect changes in IOP in healthy eyes, but leads to an increase in IOP in glaucoma and OH (35, 36). Caffeine use is associated with its effect on blood pressure, and hence the likely concern that it will lead to a spike in IOP. Caffeine reduces aqueous humor drainage by decreasing the tone of smooth muscles through the blockade of adenosine receptors, which leads to the closure of pores on the trabeculum (37). However, other possible mechanisms by which caffeine may affect IOP and thereby increase the risk of POAG are not well understood. Caffeine may increase IOP by increasing the production of aqueous humor, by inhibition of phosphodiesterase, which leads to the maintenance of high intracellular levels of cAMP in the ciliary body. Caffeine acutely causes a spike in arterial pressure. The elevated arterial pressure increases the hydrostatic pressure, which in turn creates aqueous humor from the plasma in the capillary network of the ciliary processes, potentially leading to IOP spikes. Caffeine also lowers blood flow to the macula, optic nerve, and choroid, which can make the optic nerve more sensitive to IOP spikes. This effect is proven to be more pronounced in younger women. These theoretical considerations, however, have no foundation in the proven practical impact of caffeine on IOP, since one cup of coffee (caffeine 182mg) temporarily leads to an increase in IOP. Still, this change is too small (approximately 1 mmHg) to affect the onset or progression of glaucoma (37).

Caffeine consumption does not increase the risk of glaucoma except in people who have a genetic burden or advanced stages of glaucoma (38). However, it is proven that people who drink more than 3 cups of coffee per day (caffeine > 500 mg) have an increased risk of PEX glaucoma (37).

Valuable data when discussing the connection between caffeine and glaucoma are the positive aspects of regular caffeine consumption, which have been proven. 3-Methyl-1,2-cyclopentanedione, one of the active ingredients in coffee, has a proven antioxidant effect, which may also be related to the health of the optic nerve (39).

Misconception number 8 - Blue eyes are more susceptible to glaucoma

Eyes with light irises (blue or green) have less pigment, and hence the belief that they are more susceptible to diseases. However, from a glaucomatological point of view, the opposite is correct. There is a modest but statistically significant association between a darker iris and IOP level (40). A darker, heavily pigmented iris usually indicates a thicker iris, which can lead to a narrower chamber angle and a higher probability of angle closure glaucoma (41). On the other hand, people with lighter eye color and myopia have a higher risk of developing a variant of pigmentary glaucoma, which is proven to have a genetic basis (42).

Misconception number 9 - All activities are allowed for people with glaucoma

Lifestyle and activities that are recommended or prohibited for people with glaucoma have been well studied. For people with POAG and those with OH, there are generally no limitations in daily activities (43). But, physical activity that should be avoided is anything done upside down or with the head below the level of the heart (handstands, some yoga exercises), which causes the IOP to jump as much as 2 or 3 times more than usual (44). Holding your breath during exercise also causes a drastic jump in IOP (Valsalva). This is notable during GAT, and that is, we ask our patients to breathe normally during IOP taking. If a person engages in weightlifting, it is advised to use smaller weights and more repetitions. People who have pigmentary glaucoma or pigment dispersion syndrome should not engage in strenuous sports (45).

CONCLUSION

Glaucoma is a group of chronic, non-communicable diseases that cannot be prevented or cured. Effective management requires early detection, timely therapy, and lifelong follow-up. While significant advancements have been made in the diagnosis and treatment of glaucoma, persistent misconceptions among ophthalmologists must be addressed to improve patient outcomes and quality of life.

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DEVET NAJČEŠĆIH ZABLUDA U DIJAGNOSTICI I LEČENJU GLAUKOMA

Marija Božić^{1,2}

Sažetak

Dijagnostika i lečenje glaukoma su značajno napredovali u poslednjih 20 godina, ali su i dalje u svakodnevnoj oftalmološkoj praksi prisutne neke zablude u vezi sa ovom grupom bolesti. Cilj ovog preglednog rada je da se ukaže na najčešće zablude u dijagnostici i lečenju glaukoma, što bi trebalo da utiče na podizanje kvaliteta lečenja naših pacijenata. U revijskom radu su analizirani naučni radovi publikovani u PubMed bazi podataka do 30.6.2025. godine sa ključnim rečima: glaukom, pogrešno shvatanje, merenje intraokularnog pritiska.

Ključne reči: glaukom, dijagnoza, lečenje, zablude

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Neke od najčešćih zablude u dijagnostici i lečenju glaukoma su u vezi sa načinom merenja intraokularnog pritiska, vremenom kada se merenje obavlja, oduzimanjem/dodavanjem vrednosti prema nomogramu za pahimetriju, zabrani unosa kofeina kod glaukoma i preporukama za fizičku aktivnost kod svih oblika glaukoma.

Iako je postignut značajan napredak u dijagnostici i lečenju glaukoma, i dalje postoje određene zablude među oftalmolozima koje se moraju rešiti kako bi se poboljšali ishodi lečenja i kvalitet života pacijenata.

CASE REPORT

Snakebite with a fatal outcome in Western Serbia: a rare case report

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Summary

Introduction: Snakebites can lead to a wide range of clinical manifestations, from local tissue effects to systemic complications, including rhabdomyolysis, coagulopathy, acute kidney injury, neurological, and cardiac events. Although snakebites in Western Serbia are generally not a major public health issue, severe cases may occur, with potentially fatal outcomes.

Patient review: We report the case of a 71-year-old man bitten by *Vipera ammodytes* in Bajina Bašta, Western Serbia. He was presented with fatigue, vomiting, and swelling of the left hand one hour after the bite. His past medical history included diabetes mellitus, ischemic stroke, and cerebral aneurysm repair. On admission, he was hypotensive and dehydrated, with a local hematoma and thrombocytopenia. He received antivenom, corticosteroids, dopamine, antibiotics, and supportive care. Despite initial stabilization, the patient developed acute respiratory distress, acute kidney injury, hypotension, and peripheral cyanosis, requiring mechanical ventilation and dual inotropic support. Laboratory findings indicated coagulopathy and rising markers of organ injury. Despite intensive management, the patient succumbed to complications.

Conclusion: This represents the first reported fatal snakebite in Western Serbia in the past 30 years. The primary pathogenic mechanism was likely disseminated intravascular coagulation triggered by the venom. This case highlights the importance of prolonged clinical and laboratory monitoring, timely supportive therapy, and awareness of potentially severe systemic complications following snakebites.

Keywords: Cardio- and cerebrovascular damages, comorbidities, diabetes mellitus, nose-horned viper (*Vipera ammodytes*), snakebite envenoming

INTRODUCTION

Clinical manifestations of snakebites may be limited to local effects, but can also be systemic and potentially fatal. Systemic manifestations include rhabdomyolysis, coagulopathy, acute kidney injury, stroke, and neurological and cardiac complications (1). Autopsies of individuals who died following snakebite have revealed pathological changes in nearly all organ systems (2). Locally, envenomation may cause hemorrhage, an inflammatory reaction characterized by swelling and erythema, and tissue necrosis (3).

Snakebites in the Western Serbia region do not constitute a major public health problem. Between 2006 and 2018, no fatal outcomes were recorded in this region, although some patients presented with severe clinical manifestations (4, 5).

CASE REPORT

A 71-year-old man was admitted to the Department of Infectious and Tropical Diseases at the General Hospital in Užice in July 2024 due to marked fatigue, vomiting, and pain with swelling of the left hand. His symptoms developed following a snakebite, which had occurred one hour before admission. The bite site was located on the dorsum of the left hand, between the metacarpophalangeal joints of the second and third fingers. Pain and swelling developed immediately after the bite. The patient was transported by ambulance with a medical escort. During transport, he received 500 mL of normal saline and 40 mg of methylprednisolone. The snake was located in a metal candle box at a cemetery in the small town of Bajina Bašta, located 45 km from Užice. The patient described the snake as brownish, with a nasal horn-like projection and approximately 50 cm in length.

The patient's past medical history was significant for diabetes mellitus, treated for seven years with the oral antidiabetic agent metformin. The patient had previously had an ischemic stroke and surgery for a cerebral aneurysm, with complete recovery.

On admission, the patient was notably pale, weak, and dehydrated, afebrile, and hypotensive (BP 80/40 mmHg), with normal cardiac and pulmonary auscultation and an unremarkable abdominal examination. His left hand was swollen with a hematoma on the dorsum, accompanied by swelling and lividity of the second and third fingers. Laboratory findings revealed thrombocytopenia (Table 1).

The patient immediately received antivenom, dopamine support, methylprednisolone, tetanus prophylaxis, and antibiotic therapy.

Electrocardiogram (ECG) showed sinus rhythm, left axis, frequency about 100/min, QS in D3, AVF, reduced R in the entire precordial region, and signs of left bundle branch block (LBBB) (Figure 1).

Table 1. Laboratory analyses of the patient (values higher than normal are given in boldface, and values lower than normal are italic)

Parameters (normal range)	On admission	8h later
Red blood cells ($4 - 5.9 \times 10^{12}/L$)	5.1	4.8
White blood cells ($4 - 10 \times 10^9/L$)	13.2	12.7
Neutrophil count ($1.9 - 8.0 \times 10^9/L$)	9.6	9.7
Eosinophil count ($0.0 - 0.8 \times 10^9/L$)	0.8	1.2
Platelet count ($150.0 - 450.0 \times 10^9/L$)	93	153
Prothrombin time (10.0 – 14.5 sec)	13.5	16.8
Activated partial thromboplastin time (24.3 – 35.0 sec)	28.3	42.5
Fibrinogen (2.0 – 4.0 g/L)		1.6
Blood sugar (4.1 – 5.9 mmol/L)	13.1	7.1
Blood urea nitrogen (2.8 – 7.2 mmol/L)	7.0	10.9
Serum creatinine (59.0 – 104.0 Umol/L)	127.9	268.7
Alanine aminotransferase (< 45 U/L)	22	37
Aspartate aminotransferase (15.0 – 60.0 U/L)	14	84
Sodium (132 – 146 mmol/L)	141	145
Potassium (3.5 – 5.5 mmol/L)	4.0	5.2
Lactate dehydrogenase (208.0 – 378.0 U/L)	552	568
Creatine kinase (32.0 – 294.0 U/L)	169	1,092
Creatine kinase-MB (<24 U/L)	49	37
Blood proteins (57.0 – 82.0 g/L)	66	52
Albumin (35.0 – 52.0 g/L)	39.2	31.8
C-reactive protein (< 5 mg/L)	14.7	63.9
Troponin (0.00 – 0.07 ng/mL)		3.52
D-dimer (0 – 230 ng/ml)		77,507

Three hours later, the patient was in stable general condition, with BP 115/70 mmHg and a urine output of 300 mL.

Eight hours after admission, the patient developed dyspnea, hypotension, and peripheral cyanosis, with a drop in oxygen saturation (SpO₂ 70%). Oxygen support was provided at 15 L/min via an oxygen mask. Cardiac rhythm was regular, heart rate 130/min, BP 70/40 mmHg. Auscultation revealed late inspiratory crackles bilaterally at the lung bases.

Chest X-ray showed bilateral basal pulmonary infiltrates. Repeated ECG did not show significant changes compared to the previous recording (Figure 2).

Treatment was continued in the intensive care unit, where the patient received non-invasive ventilation (NIV). Dual inotropic support (dobutamine and nor-epinephrine) was initiated, and stimulated diuresis was monitored via a urinary catheter. Due to a further drop in oxygen saturation (SpO₂ 60%), the patient was intubated and placed on mechanical ventilation in PC-SIMV mode with FiO₂ 100%. BP was 86/50 mmHg, and urine output was 100 mL. No new changes were observed on the ECG (Figure 3). Shortly thereafter, the patient experienced a fatal outcome. Approximately 11 hours elapsed from the time of the bite to the fatal outcome.

The patient's family declined a requested autopsy.

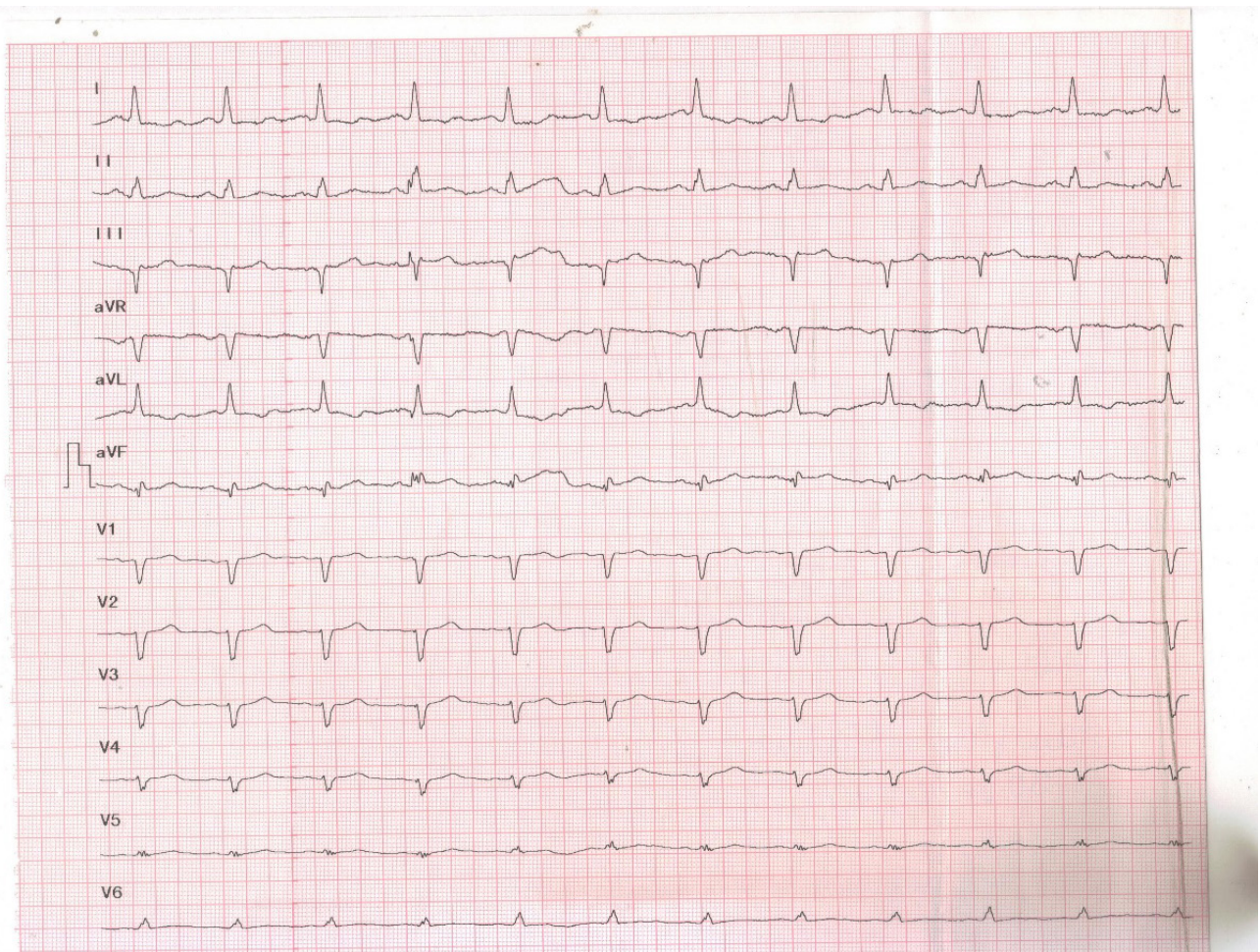


Figure 1. Electrocardiogram (ECG) of the presented patient on admission

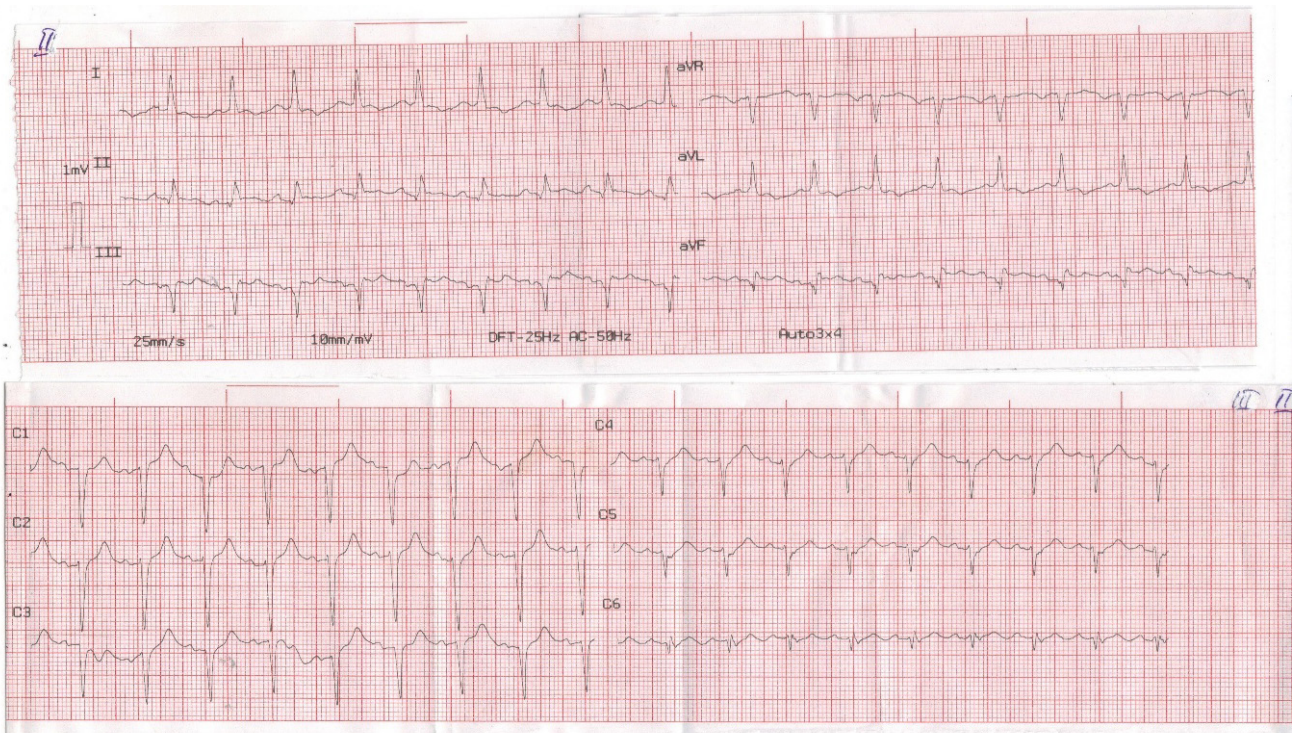


Figure 2. Electrocardiogram (ECG) of the presented patient at 7 pm

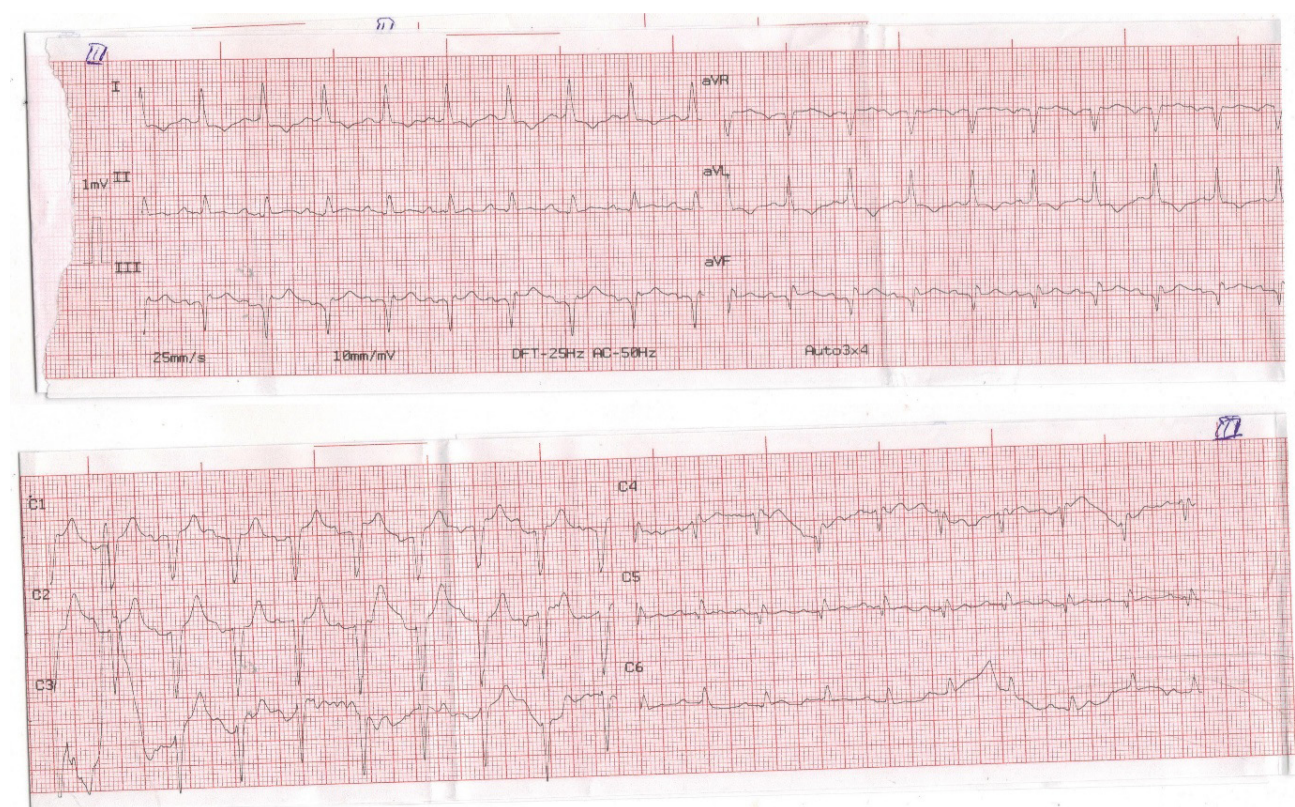


Figure 3. Electrocardiogram (ECG) of the presented patient at 11.40 pm

DISCUSSION

The clinical course following a venomous snakebite depends on the composition of the snake's toxins, the development of inflammation, and the host's immune defense mechanisms (6).

The toxin exhibits neurotoxic, cytotoxic, or hemotoxic effects, causing damage to multiple tissues and organs. Death most commonly occurs due to paralysis of the respiratory muscles. Insufficient gas exchange in the lungs leads to organ hypoxia. Clinically, this most often manifests as asphyxia, hypoxic encephalopathy, and cardiomyopathy (7).

We presented the first fatal outcome following a snakebite reported in Western Serbia in the past 30 years. The snake was identified as *Vipera ammodytes*.

The snake venom is composed predominantly of proteins and peptides. The chemical composition of the *Vipera ammodytes* venom explains the frequent occurrence of coagulopathy and multi-organ hemorrhages (8). Phospholipase A₂, metalloproteinases, and L-amino acid oxidase from snake venom induce inflammation. Consequently, levels of proinflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, and tumor necrosis factor-alpha (TNF-α) increase. The immune response occurs both locally and in all organs and tissues reached by the venom (9).

A serine protease is most commonly responsible for disrupting hemostatic mechanisms in mammals. This component of snake venom induces vasoconstriction, enhances platelet aggregation, and triggers fibrinolysis (10).

Upon admission, our patient was in a state of hypovolemic shock. A stable condition was initially achieved with the administered therapy.

Although he did not experience chest pain, the initial impression of subsequent deterioration was acute coronary syndrome, considering the risk factors of diabetes and a history of circulatory disorders (11). This was supported by elevated troponin and aspartate aminotransferase (AST) levels. Cardiotoxicity typically manifests as myocardial infarction, even in the absence of ECG changes (12). Liblik and colleagues reported that in approximately 25% of analyzed cases, the cardiotoxic effects of the toxin resulted in cardiac arrest, myocardial infarction, myocarditis, or ventricular dysfunction (1). Mast cell degranulation and the release of inflammatory mediators alter all cardiac structures (13).

A reaction to snake venom or to antivenom may also present as Kounis syndrome, accompanied by eosinophilia, as in our patient (14, 15). Of the three described variants of this syndrome, our patient may have had type I, with normal ECG features, cardiac enzymes, and troponin (16). ECG changes may develop later in the course of the disease (17). Our patient showed an increase in creatine kinase (CK) levels alone, not in the CK-MB fraction. AST was progressively rising, without any ECG changes. Diagnostic evaluation in our case was limited by the fact that the patient's condition deteriorated suddenly at around 22:30, when no on-call cardiologist was available.

Coronary angiography was not performed, although this finding can also appear normal (18).

As the clinical condition deteriorated, laboratory tests showed markedly elevated D-dimer levels, indicating the development of coagulopathy. Disseminated intravascular coagulation (DIC) is a possible consequence of snake venom activity (19, 20). Supporting the suspicion of DIC were the prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT), as well as decreased fibrinogen levels.

The changes observed in our patient's pulmonary parenchyma may be due to the strong toxic effects of snake venom. Infiltrates, edema, hemorrhages, and acute respiratory distress syndrome have all been described (21). In our case, computed tomography of the lungs and pulmonary arteries was not performed.

The acute kidney injury in our patient was caused by both the direct toxic effect of the venom and the development of DIC. Severe cases of acute kidney injury have been reported. The most common pathological findings are acute tubular necrosis and interstitial nephritis (22).

CONCLUSION

The limitations of the reported case of a fatal snakebite include the lack of imaging diagnostics and post-mortem examination. Based on the clinical course and laboratory

parameters, we hypothesize that the primary pathogenic mechanism was disseminated intravascular coagulation. Given that such cases are rare in Western Serbia, awareness of the need for prolonged clinical and laboratory monitoring of these patients, as well as timely access to additional diagnostic tools, is essential.

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Ethical approval: The use of clinical data was approved by the Užice Health Centre ethics committee (Decision No. 1464) on February 12, 2025.

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UJED OTROVNE ZMIJE SA FATALNIM ISHODOM U ZAPADNOJ SRBIJI: PRIKAZ RETKOG SLUČAJA

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Sažetak

Uvod: Ujedi otrovnih zmija mogu izazvati širok spektar kliničkih manifestacija, od lokalnih efekata na tkiva do sistemskih komplikacija, uključujući rhabdomiolizu, koagulopatiju, akutno oštećenje bubrega, neurološke i kardiološke poremećaje. Iako u zapadnoj Srbiji ujedi otrovnih zmija ne predstavljaju ozbiljan problem javnog zdravlja, može doći do ozbiljnih situacija, sa potencijalno fatalnim ishodom.

Prikaz pacijenta: Opisali smo slučaj sedamdesetjednogodišnjeg muškarca kojeg je ujeo poskok u Bajinoj Bašti, u zapadnoj Srbiji. Javio se sa umorom, povraćanjem i otokom leve ruke jedan sat nakon ujeda. Njegova prethodna medicinska istorija uključivala je dijabetes melitus, moždani udar i operaciju cerebralne aneurizme. Na prijemu je bio hipotenzivan i dehidriran, sa lokalnim hematomom i trombocitopenijom. Primio je protivotrov,

Ključne reči: Kardio- i cerebrovaskularna oštećenja, komorbiditeti, dijabetes melitus, poskok (*Vipera ammodytes*), ujed otrovne zmije

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kortikosteroide, dopamin, antibiotike i suportivnu negu. Uprkos početnoj stabilizaciji, kod pacijenta su se razvili akutni respiratorni distres, akutno oštećenje bubrega, hipotenzija i periferna cijanoza, što je zahtevalo mehaničku ventilaciju i dvostruku inotropnu podršku. Laboratorijski nalazi su ukazivali na koagulopatiju i porast markera oštećenja organa. Uprkos intenzivnom lečenju, pacijent je podlegao komplikacijama.

Zaključak: Slučaj koji smo predstavili prvi je prijavljen smrtonosni ujed zmije u zapadnoj Srbiji u poslednjih 30 godina. Primarni patogeni mehanizam je verovatno bila diseminovana intravaskularna koagulacija izazvana otrovom. Ovaj slučaj ističe važnost produženog kliničkog i laboratorijskog praćenja, blagovremene suportivne terapije i svesti o mogućim teškim sistemskim komplikacijama nakon ujeda zmija.

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