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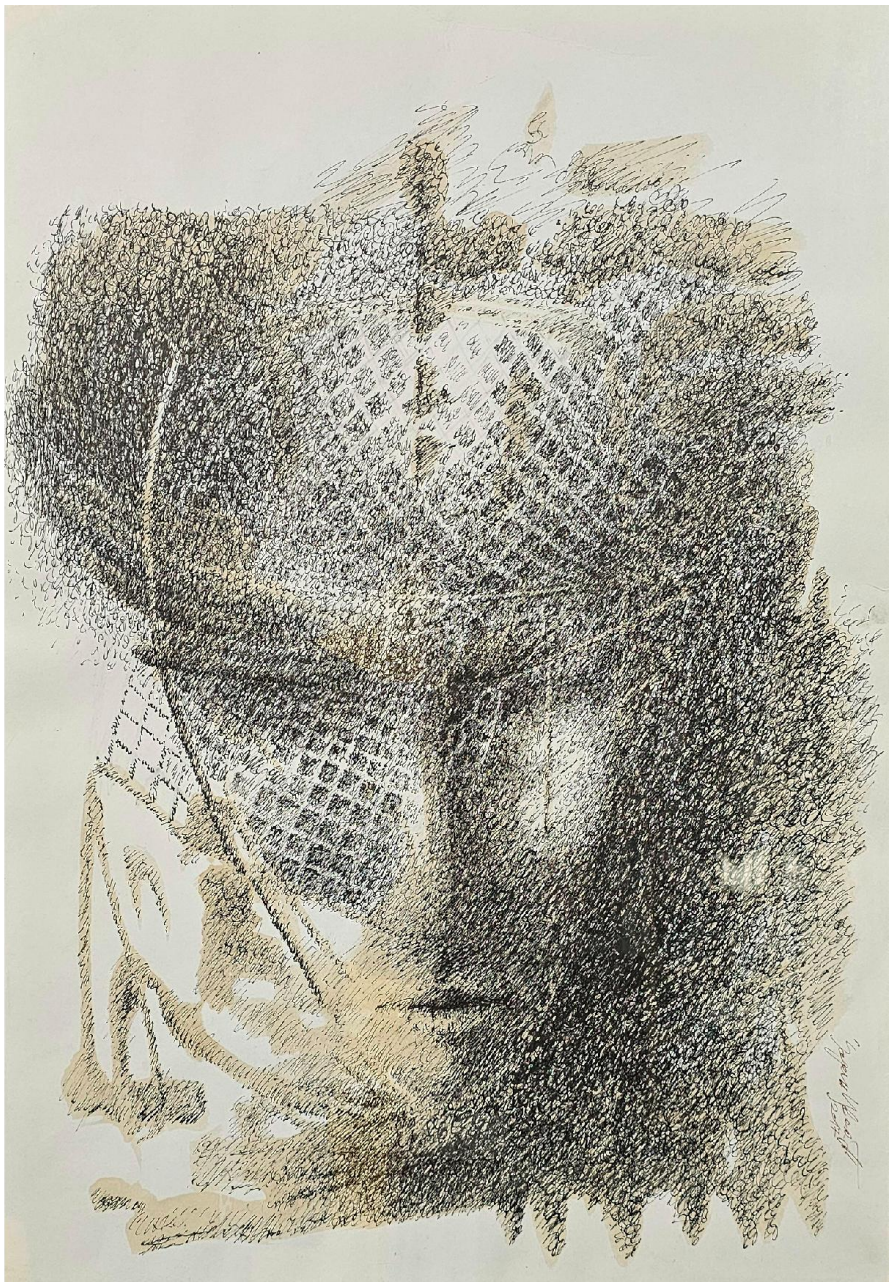

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C O N T E N T S

ORIGINAL PAPERS

REVIEW ARTICLE

CASE REPORT

HISTORY OF MEDICINE

DIAGNOSTIC VALUE OF PREOPERATIVE LABORATORY AND IMAGING PARAMETERS IN STRATIFYING APPENDICITIS SEVERITY

Srđan Milina, Branko Lukić, Marija Nikolić, Nemanja Trifunović, Milica Radivojević, Dimitrije Surla

DEPARTMENT OF GENERAL SURGERY, CLINICAL HOSPITAL CENTER "ZEMUN", BELGRADE, SERBIA

ABSTRACT: Background: Acute appendicitis is the most common intra-abdominal surgical emergency. Accurate differentiation between uncomplicated and complicated appendicitis is essential for determining optimal treatment and minimizing postoperative morbidity. While laparoscopic appendectomy remains the gold standard, conservative management is increasingly considered a viable option for uncomplicated cases. **The aim** of our study was to highlight the importance of preoperative laboratory and imaging diagnostics in differentiating the severity of intraoperative findings in acute appendicitis and optimizing therapeutic strategy. **Methods:** This retrospective study analyzed 231 patients diagnosed with acute appendicitis at the Clinical Hospital Center Zemun between December 2021 and September 2023. Patients were stratified into uncomplicated and complicated appendicitis groups based on clinical, intraoperative, and histopathological findings. Demographic, clinical, laboratory and ultrasonographic data were compared between groups. Statistical analyses were performed using SPSS version 21. **Results:** Complicated appendicitis was present in 63 patients (27.27%), who were significantly older and had longer symptom duration ($p < 0.01$). Febrile episodes, leukocytosis, and elevated CRP levels were more prevalent in the complicated group. The mean CRP concentration was significantly higher in the complicated group (109 mg/L) compared to the uncomplicated group (24.55 mg/L, $p < 0.01$). Ultrasonography showed limited ability to differentiate between disease severities. No significant association was found between diabetes mellitus and disease complexity. **Conclusion:** Our study confirms that older age, fever, prolonged symptom duration, and elevated CRP levels are key factors associated with complicated acute appendicitis. CRP stood out as the most reliable preoperative biomarker for assessing disease severity. These results emphasize the need to integrate clinical evaluation with selected laboratory markers, especially CRP to improve preoperative diagnosis and guide timely treatment.

Keywords: acute appendicitis, CRP, complicated appendicitis, diagnosis, laboratory markers

INTRODUCTION

Acute appendicitis is the most common intra-abdominal surgical emergency [1]. In Serbia, the morbidity of acute appendicitis is similar to global trends, and the overall lifetime risk is approximately 8.6% in men and 6.7% in women [2]. The diagnosis and management of acute appendicitis can be complex, as they require the exclusion of various differential diagnoses and prioritization of surgical intervention according to disease severity. Timely and accurate treatment remains essential for reducing appendicitis-related morbidity.

Distinguishing between uncomplicated and complicated appendicitis is crucial for determining the appropriate therapeutic approach [3]. Inadequate or delayed diagnosis and treatment of complicated appendicitis are associated with serious complications and postoperative morbidity, further emphasizing the importance of identifying parameters that reflect disease severity [4].

Accurate differentiation between these two forms aims to support the selection of the most appropriate therapy, while simultaneously reducing the number of unnecessary surgical interventions and the risk of associated complications. In addition to laparoscopic appendectomy, which remains the gold standard for the treatment of complicated appendicitis, a conservative approach is increasingly being used in the management of uncomplicated cases [5–7].

The diagnosis of appendicitis is primarily based on the clinical presentation, although inflammatory markers, ultrasound, and

computed tomography can contribute to diagnostic accuracy [8]. In our institution, ultrasound examination is routinely used together with the assessment of C-reactive protein (CRP) levels and white blood cell (WBC) count to facilitate diagnosis and evaluate disease severity. CT is used rarely, and only in cases where the clinical presentation and laboratory findings are not sufficiently clear. C-reactive protein (CRP) is an inflammatory marker that has been identified in several studies as an independent predictor of complicated appendicitis [9,10].

The main objective of this study is to determine whether CRP can be used to distinguish between complicated (gangrenous or perforated) and uncomplicated appendicitis.

MATERIALS AND METHODS

This study presents a retrospective analysis of 231 patients with clinical signs of acute appendicitis who were hospitalized at the Department of General Surgery of the Clinical Hospital Center (KBC) Zemun between December 2021 and September 2023. All data used in the study were obtained from medical records. The patients underwent surgical treatment, either open or laparoscopic appendectomy, and the diagnosis of acute appendicitis was confirmed by postoperative histopathological examination of the removed appendix.

Based on clinical, intraoperative, and histopathological findings, the patients were divided into two groups: those with uncomplicated appendicitis and those with complicated appendicitis. Uncomplicated appendicitis was defined as catarrhal or phlegmonous inflammation of the appendix, whereas complicated appendicitis referred to gangrenous inflammation, with or without perforation.

In addition to analyzing and comparing C-reactive protein levels, white blood cell counts, and ultrasound findings, the study also assessed various clinical and demographic parameters. These included symptom duration, the presence or absence of febrile episodes, and the existence of comorbidities. Basic demographic characteristics such as patient sex and age were also included to evaluate their potential impact on clinical presentation and disease course. A body temperature higher than 37.4°C was considered significant.

Every clinical suspicion of appendicitis was further confirmed by mandatory laboratory analyses and ultrasound examination to increase diagnostic accuracy and guide further management. A positive ultrasound finding was established based on the identification of one or more of the following criteria: presence of free intraperitoneal fluid, regional lymphadenopathy, and/or an increased appendiceal diameter with thickening of the appendiceal wall.

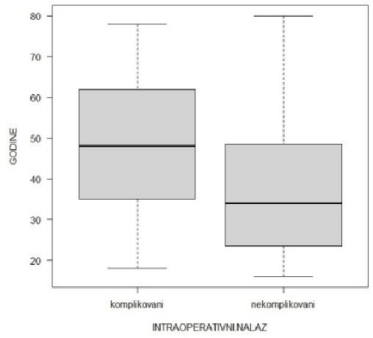
Statistical analysis was performed using SPSS software, version 21. Variables between the two patient groups were compared using the Mann–Whitney U test for numerical data and the chi-square test for categorical data. Statistical significance was defined as a p-value of less than 0.05..

RESULTS

The sample of 231 patients diagnosed with appendicitis during the study period was divided into two groups. The first group consisted of 168 patients (72.73%) with uncomplicated appendicitis, while the second group included 63 patients (27.27%) with complicated appendicitis. The overall patient population comprised 110 men (47.62%) and 121 women (52.38%). Among patients with complicated appendicitis, 34 (54%) were men and 29 (46%) were women, whereas in the group with uncomplicated appendicitis, 76 (45.2%) were men and 92 (54.8%) were women. No statistically significant difference in sex distribution was observed between the two groups ($p = 0.242$).

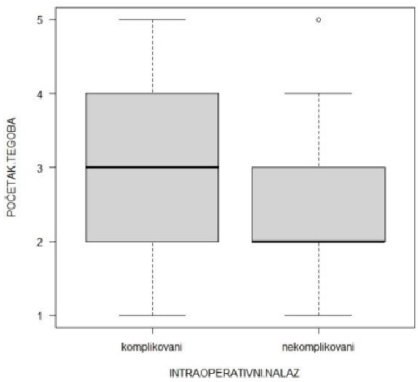
The mean age of our patients was 40.31 ± 17.06 years, with the youngest patient being 16 and the oldest 80 years old. In the subgroup of patients with complicated appendicitis, the median age was 47 years. In contrast, patients with uncomplicated appendicitis were significantly younger, with a median age of 34 years, representing a statistically significant difference ($p < 0.01$).

Figure 1. The box-plot diagram illustrates the relationship between patient age and intraoperative findings in complicated and uncomplicated appendicitis. The diagram indicates a statistically significant difference in the severity of intraoperative findings between younger and older patients. The central line within each box represents the median age, while the edges of the box denote the first and third quartiles.



In most patients with complicated inflammation, symptoms appeared within 24 to 48 hours prior to establishing the preoperative diagnosis. In contrast, patients with uncomplicated inflammation most commonly experienced symptoms for less than 24 hours before arriving at the hospital. This difference in symptom duration between the two groups was statistically significant ($p < 0.01$), indicating an association between longer symptom duration and the development of complicated disease. These findings highlight the importance of early clinical assessment and intervention to reduce the risk of complications..

Figure 2. The box-plot diagram illustrates the relationship between symptom duration and intraoperative findings in complicated and uncomplicated appendicitis. The diagram shows that longer symptom duration is associated with a higher incidence of complicated intraoperative findings. The central line within each box represents the median duration, while the edges of the box denote the first and third quartiles..



A total of 63 patients (27.27%) experienced episodes of elevated body temperature during the course of their symptoms, of whom 26 had complicated and 37 had uncomplicated appendicitis. Considering the difference in group size, the difference in the presence of fever between the groups was statistically significant ($p < 0.01$).

Regarding comorbidities, a total of 7 patients (3.02%) were diagnosed with diabetes

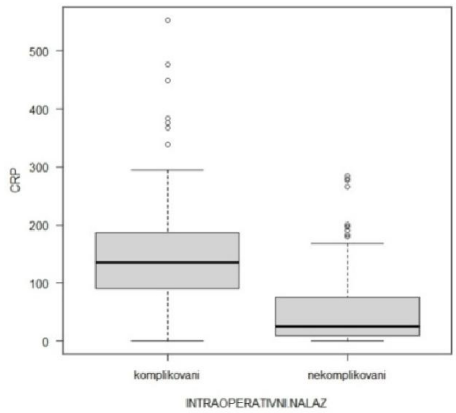
mellitus. Of these, 5 patients were in the uncomplicated appendicitis group, while 2 patients were in the complicated appendicitis group. Statistical analysis did not show a significant difference in the prevalence of diabetes between the groups, suggesting that in this sample, the presence of DM did not significantly influence the likelihood of developing complicated appendicitis.

Ultrasound examination demonstrated findings indicative of acute appendicitis in 134 patients (58.01%), while 97 patients (41.99%) had no ultrasonographic evidence of the disease. Among patients with positive ultrasound findings, the prevalence of complicated versus uncomplicated appendicitis was assessed. Although a higher percentage of patients with positive findings had complicated appendicitis, the difference between the groups was not statistically significant ($p = 0.134$).

In this study, the mean white blood cell count in peripheral blood was 12.61×10^9/L ± 4.64×10^9/L, indicating that most patients presented with leukocytosis. In patients with uncomplicated appendicitis, leukocyte values ranged from 2.2×10^9/L to 25.2×10^9/L, with a mean of 11.8×10^9/L. In the complicated appendicitis group, leukocyte counts ranged from 4.3×10^9/L to 27.8×10^9/L, with a mean value of 14.4×10^9/L. Comparative analysis of this inflammatory marker between the two groups demonstrated a statistically significant difference ($p < 0.01$).

C-reactive protein (CRP) values among patients varied considerably, with a standard deviation slightly lower than the mean, amounting to 66.62 ± 65.60 mg/L. CRP concentrations ranged from 0.2 mg/L to 265.4 mg/L. The median CRP level in patients with uncomplicated appendicitis was 24.55 mg/L, which was significantly lower than the median of 109 mg/L observed in patients with complicated appendicitis..

Figure 3. The box-plot diagram illustrates the relationship between C-reactive protein (CRP) levels and intraoperative findings in appendicitis. Higher CRP levels are associated with more severe, complicated forms of appendicitis. The central line within each box represents the median CRP value, while the edges of the box denote the first and third quartiles..



DISCUSSION

Preoperative differentiation between complicated and uncomplicated acute appendicitis remains a major clinical challenge, particularly when clinical findings are unclear and laboratory tests lack sufficient specificity [11,12]. This diagnostic uncertainty contributes to a substantial rate of misdiagnosis, which, according to several studies, ranges between 12% and 30% [2,8,10]. Such errors may lead to unnecessary surgical intervention or delays in treatment, thereby worsening clinical outcomes. Therefore, improving diagnostic tools and criteria is essential for optimizing disease management and reducing negative appendectomies and complications.

Traditionally, early appendectomy has been recommended for uncomplicated appendicitis to prevent rupture [13]. However, recent randomized studies [13–15] and meta-analyses [16,17] show that nonoperative antibiotic treatment may be successful in

carefully selected patients with uncomplicated appendicitis. According to the updated guidelines of the World Society of Emergency Surgery (WSES), established at the Jerusalem Consensus Conference in 2020, antibiotic therapy is recommended as a safe and effective alternative to surgery in patients with uncomplicated appendicitis without appendicolithiasis. It is important to note that unrecognized perforation may lead to severe complications such as abscess formation and purulent peritonitis [19]. The reported perforation rate in acute appendicitis ranges between 20% and 34% [20–22]. Among patients treated nonoperatively, it should be emphasized that there is an approximately 39% risk of recurrence within 5 years [14,18].

Laparoscopic appendectomy remains the gold standard for the management of suspected complicated appendicitis [23]. The need for a more invasive approach is justified by the serious complications that may arise, including infection, ileus, intra-abdominal abscess, and fistula formation [24–26]. Delaying surgery increases the risk of life-threatening conditions and rehospitalization [27].

Demographic characteristics, particularly age and sex, have been identified as significant factors in the development of complicated appendicitis. Birben et al. reported a higher incidence of complicated appendicitis in older and male patients [28]. Our study did not demonstrate a statistically significant sex difference, but patients with complicated appendicitis were significantly older. This may be explained by age-related declines in immune function and physiological reserves. Numerous studies report similar findings [2,3,6,29,30].

A long interval between symptom onset and diagnosis is directly associated with the development of complications. Our study confirms that longer symptom duration is a predictor of complicated disease, consistent with previous research [2,3,8,23,30,31].

Elevated temperature (≥37.4°C) has been shown to be a clinically useful indicator of complicated appendicitis [30,31,33]. Our findings, as well as the study by Akai et al. [34], confirm the significance of body temperature in assessing disease severity.

Although diabetes is associated with immune dysfunction and a more severe course of appendicitis, our study did not show a significant association, likely due to the small number of diabetic patients and better disease control in our institution [2,35].

Ultrasound is frequently the first diagnostic modality, but it is limited by subjective interpretation and patient-related factors [3,36,37]. Our results confirm that ultrasound alone is insufficient for distinguishing complicated from uncomplicated disease and should be used in combination with clinical evaluation and laboratory markers.

Leukocytosis is an important but nonspecific marker. Our results confirm its correlation with complicated appendicitis, although it lacks sufficient standalone predictive value [4,7,8,27,38].

CRP, as an acute-phase protein, exhibits proportional increases with the severity of inflammation [3,27,29,30,31,33,38,39]. Our study confirmed its markedly elevated levels in complicated cases (median 109 mg/L), further supporting its diagnostic utility..

CONCLUSION

Our study confirms that older age, elevated body temperature, longer symptom duration, and increased CRP levels are key factors associated with complicated acute appendicitis. CRP emerged as the most reliable preoperative biomarker for assessing disease severity. These findings emphasize the need to integrate clinical evaluation with selected laboratory parameters, particularly CRP, to ensure a more accurate preoperative diagnosis and the implementation of timely therapeutic strategies.

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THE ROLE OF CLINICAL AND LABORATORY PARAMETERS IN RISK ASSESSMENT OF PATIENTS WITH CARDIOVASCULAR DISEASES

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SUMMARY: Introduction: Cardiovascular and metabolic diseases represent a significant challenge in modern healthcare due to their high mortality rates and impact on quality of life. Accurate risk assessment is essential for therapy personalization and the prevention of complications, encompassing clinical, inflammatory, and hemostatic risk factors. The aim of this study was to investigate the role of clinical and laboratory parameters in risk assessment among patients with cardiovascular diseases, with a particular focus on inflammatory and hemostatic indices such as the Neutrophil-Lymphocyte Ratio (NLR), Mean Platelet Volume (MPV), and the Red Cell Distribution Width to Platelet Ratio (RPR). Subjects and Methods: The study included 311 patients with cardiovascular diseases. Retrospective and prospective methods were used to collect clinical and laboratory data, including body mass index, blood pressure, and lipid profile. Statistical analysis comprised factor analysis and multiple linear regression. Results: The study demonstrated that clinical and laboratory parameters, including LDL cholesterol, as well as inflammatory-hemostatic indices such as MPV and RPR, were significantly associated with the presence and combinations of cardiovascular diseases. The RPR index was highest in patients with hypertension and stents ($p < 0.001$), indicating increased procoagulability and inflammatory status in these groups. Factor analysis identified three main factors, of which the age-hematological factor was the only one showing a statistically significant association with cardiovascular diseases ($p = 0.008$), confirming its predictive value. Conclusion: These findings suggest that the integration of clinical and laboratory indicators, with a particular emphasis on inflammatory-hemostatic indices and age-hematological variables, allows for earlier identification of high-risk patients and provides a foundation for the development of personalized therapeutic strategies.

Keywords: clinical parameters, laboratory parameters, cardiovascular diseases

INTRODUCTION

Cardiovascular (CVD), as well as metabolic diseases, represent one of the most significant challenges in modern healthcare, due to their high mortality rates and severe impact on patients' quality of life. These conditions encompass a wide range of disorders, including coronary artery disease (CAD), arterial hypertension (HTN), and dyslipidemia (DLD), which often coexist and influence each other [1]. According to the World Health Organization, cardiovascular diseases are the leading cause of death globally, further emphasizing the need for effective prevention and treatment strategies [2].

Cardiometabolic syndrome represents a contemporary epidemic that can predict overall and cardiovascular mortality, the incidence and progression of coronary and carotid atherosclerosis, as well as sudden death, independently of other cardiovascular risk factors [3]. A significant increase has recently been observed in the incidence of comorbidities between metabolic disorders and cardiovascular diseases, particularly in populations at risk for metabolic syndrome or pre-metabolic syndrome [4]. Individuals with metabolic syndrome (MetS) have a threefold higher relative risk of myocardial infarction or stroke, a twofold higher risk of CVD or death from such events, and a fivefold higher risk of developing type 2 diabetes mellitus (T2DM) in both sexes compared to those without the syndrome [5–7].

Research indicates that nearly half of patients with coronary artery disease meet the criteria for metabolic syndrome [5,7,8]. One study demonstrated a clear association between cumulative exposure to metabolic disorders and

myocardial infarction, highlighting the importance of managing metabolic disorders for CVD prevention [9]. Another study revealed a positive correlation between cumulative metabolic burdens and the risk of developing atrial fibrillation (AF) [10]. Promoting an active lifestyle can improve metabolic disorders in some patients and aid in blood pressure control [11]. Both individual components of MetS and MetS as a whole increase the risk of heart failure (HF) and ischemic stroke [12]. Reverse causation factors could not be adequately accounted for in observational studies, and therefore the causal relationship between metabolic disorders and various CVDs remains uncertain [13].

The importance of risk assessment in patients with cardiovascular and metabolic disorders lies in the ability to identify high-risk individuals, allowing timely intervention and tailored therapeutic strategies. Risk stratification using SCORE-2 (Systematic Coronary Risk Evaluation) enables the estimation of 10-year risk for cardiovascular events, such as myocardial infarction and stroke. This tool takes into account factors including age, sex, blood pressure, cholesterol levels, smoking status, and the presence of diabetes. In modern medical practice, risk assessment using SCORE-2 and SCORE2-OP for older patients [14,15] represents an essential component of clinical decision-making. This approach facilitates the development of individualized therapy and prevention plans, thereby significantly reducing the overall risk of serious cardiovascular events. The use of this tool allows for more accurate identification of high-risk patients and the implementation of appropriate interventions, contributing to improved health outcomes.

As noted, risk assessment in patients with cardiovascular and metabolic diseases constitutes a fundamental aspect of contemporary clinical medicine, given the prevalence of these conditions and their significant contribution to morbidity and mortality worldwide. Cardiovascular diseases, including ischemic heart disease and cerebrovascular disorders, are frequently associated with metabolic disturbances such as diabetes mellitus (DM) and metabolic syndrome (MetS). This association is reflected in shared pathophysiological mechanisms, which further complicates diagnosis and management. Clinical parameters, including—but not limited to—blood pressure, lipid profile, blood glucose levels, body mass index (BMI), and inflammatory markers, serve as the basis for risk stratification and the personalization of therapeutic strategies [16].

Analysis of these interrelated parameters allows for the identification of individuals with multiple risk factors, who are often classified as very high risk, which is crucial for the implementation of preventive interventions. The role of each of these clinical indicators cannot be overstated, as they not only help assess the patient's current health status but also enable the prediction of future cardiovascular events. Accordingly, monitoring the complexity of these parameters and their interactions can significantly enhance prevention and treatment strategies.

Age is the strongest non-modifiable risk factor for CVD. The increase in cardiovascular risk is continuous and progressive in both men and women. However, the transition to the high-risk category for the development of cardiovascular disease appears to occur at specific ages for each sex [17]. Considering a risk estimated at over 20% for 10 years for the composite outcome of myocardial infarction, stroke, and all-cause mortality, the transition to high risk occurred at 48 years for men and 54 years for women. When the broader definition of CVD included revascularization, the age of transition decreased to 41 and 48 years for men and women, respectively. The transition from low to moderate risk occurred at 35 and 45 years for men and women, respectively, according to the broader definition [17]. In the general population, the incidence of myocardial infarction is higher in men than in women, with age-adjusted risk coefficients [17].

A study by Cai et al., which included 10 observational studies with a total of 166,027 patients with type 1 diabetes mellitus (T1DM), concluded that among these patients, the relative risk of mortality was 5.09; for coronary artery disease 9.38; myocardial infarction 6.37; atrial fibrillation 1.36; and stroke 4.08. This further indicated that the relatively increased risk for CAD, CVD, myocardial infarction, and stroke was higher in women with T1DM [18]. Thus, women with type 1 diabetes have a 50% higher relative risk for fatal coronary events

compared to men. This phenomenon may be partly explained by a less favorable cardiovascular risk profile in women, associated with hypertension (HTN) and hyperlipidemia. It is also important to consider whether hormonal changes during menopause further contribute to this increased risk, given that decreased estrogen levels may negatively affect women's cardiovascular health.

Hypertension (HTN) is a well-established risk factor for cardiovascular diseases (CVD) and stroke-related mortality. Isolated systolic hypertension is a major risk factor for coronary artery disease (CAD) across all age groups and in both men and women [19,20].

LDL cholesterol is one of the most important modifiable risk factors for cardiovascular morbidity and mortality. Data from the supplementary observational MRFIT study [21], conducted in the pre-statin era, showed that among 342,815 middle-aged men in the United States (of whom 5,163 had diabetes mellitus, DM) followed for 16 years, the absolute adjusted risk of death from CVD, stratified by LDL cholesterol level, was significantly higher in patients with DM. Specifically, the risk of cardiovascular mortality was between 2.83 and 4.46 times higher in patients with DM compared to those without DM, highlighting the importance of LDL stratification as a key risk factor in this population [34]. LDL cholesterol is commonly stratified into the following categories: optimal (<100 mg/dL), near optimal (100–129 mg/dL), borderline high (130–159 mg/dL), high (160–189 mg/dL), and very high (≥190 mg/dL). Patients with DM often present with elevated LDL cholesterol levels, further increasing their risk for developing cardiovascular disease. Regular monitoring and management of LDL cholesterol in these patients are recommended to reduce the risk of cardiovascular events. The MRFIT study [21] also demonstrated that the increase in CVD mortality was disproportionately higher in patients with DM, suggesting that cholesterol is a strong and independent risk factor for CVD mortality, potentiated by DM. However, the question arises: which is the stronger risk factor—DM or LDL cholesterol? While both factors play a significant role, research suggests that DM may exert a stronger influence on cardiovascular outcomes, particularly when considering additional factors such as inflammation and metabolic disturbances present in patients with DM [35]. According to Zhang et al., chronic low-grade inflammation of the vascular intima in patients with type 2 diabetes further increases the risk of CVD, while Chen et al. highlighted that GLP-1 agonists significantly reduce cardiovascular events in this population, suggesting that DM has a stronger impact on cardiovascular outcomes [35,36]. Considering LDL reduction with statins, the study by Hodkinson et al. showed that lowering LDL by 1 mmol/L with a statin reduces relative CVD risk by one-fifth. The study also indicates that this linear phenomenon can occur similarly at any baseline LDL level, at least down to 1.293 mmol/L (50 mg/dL) [22]. In patients with DM, statins promote a proportional reduction of 9% in all-cause mortality ($p=0.02$) and 21% in major vascular events ($p<0.0001$) per mmol/L reduction in LDL-C. Additionally, there is a significant reduction in acute myocardial infarction ($p<0.0001$), coronary revascularization ($p<0.0001$), and stroke ($p<0.0002$).

The aim of this study is to investigate the relationship between clinical and laboratory parameters, with a particular focus on inflammatory-hemostatic indices, in predicting cardiovascular risk. This work also seeks to identify key factors that may facilitate the early recognition of high-risk patients, enabling timely intervention and tailored therapy..

MATERIAL AND METHODS

This research was conducted as a combined retrospective-prospective, cross-sectional, longitudinal cohort study at the Pirot Health Center, during the period from January 1, 2024, to June 1, 2024. In the retrospective part, patients' electronic health records were analyzed to collect data on demographic characteristics, smoking status, pharmacotherapy over the previous six months, and laboratory results not older than six months. In the prospective part, new data on anthropometric measurements and blood pressure values were systematically collected during each patient visit. The study was conducted in accordance with the Helsinki Declaration, and ethical approval was obtained

from the Ethics Committee of the Pirot Health Center, Pirot (reference number: 02-15/EO).

The study included 311 patients over 40 years of age with previously diagnosed type 2 diabetes mellitus (T2DM) and/or cardiovascular diseases (coronary artery disease, arterial hypertension, angina pectoris, atrial fibrillation, or implanted stent). All patients had diabetes for more than five years, ensuring the relevance of the results. Exclusion criteria included patients with malignancies, chronic kidney disease, acute infections, pregnant women, and those who had undergone therapy changes within the last six months. All participants provided written informed consent.

The control group consisted of patients with T2DM without clinically or instrumentally confirmed cardiovascular events (n=52), serving as a reference subgroup for comparison with other cohorts. For each patient who met the inclusion criteria and agreed to participate in the study, data were collected from electronic health records regarding age, sex, smoking status, and laboratory parameters not older than six months, including:

Hematological parameters: total leukocyte count, neutrophils, lymphocytes, erythrocytes, and platelets.

Biochemical parameters: glucose, glycated hemoglobin A1c (HbA1c), total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, urea, and creatinine.

Inflammatory markers: inflammation was assessed through the neutrophil-to-lymphocyte ratio (NLR), red cell distribution width to platelet count ratio (RPR), and mean platelet volume to platelet ratio (MPR).

Continuous variables were tested for normality using the Kolmogorov-Smirnov test. For comparisons between multiple groups, the Kruskal-Wallis test was applied (a non-parametric test that does not require normal distribution, as factor analysis was conducted in the study), followed by post hoc Mann-Whitney U tests with Bonferroni correction. Categorical variables were compared using the Chi-square test or Fisher's exact test, where applicable. To reduce dimensionality, principal component analysis (PCA) was performed, with the Kaiser-Meyer-Olkin (KMO) test (>0.50) and Bartlett's test of sphericity ($p<0.05$), Varimax rotation, and extraction of factors with eigenvalues >1.0. Predictors were assessed using binary logistic regression, and multiple linear regression was applied for modeling dependent indices MPR and RPR. All tests were two-sided, and a p-value <0.05 was considered statistically significant.

In the methodology, the parameters listed in Table 1 were used to evaluate different combinations of cardiovascular diseases and metabolic disorders. Key demographic (sex – percentage of men and women in different groups; age – mean age of patients in each group), clinical (BMI – body mass index for each group; blood pressure – systolic and diastolic values in different patient groups; smoking – percentage of smokers), and biochemical parameters (glucose and HbA1c – mean values for patients with diabetes, hypertension, and their combinations; total cholesterol, LDL-C, HDL-C – mean values for different groups; triglycerides – mean values) were analyzed for their potential significance in risk stratification.

It is important to emphasize the p-values indicating statistically significant differences between groups, as shown in the last column ("p"). These values are essential for understanding differences among patient groups with various metabolic disorders and cardiovascular diseases.

RESULTS

Table 1. Baseline clinical and biochemical data of patients with various metabolic and cardiovascular diseases, as well as their combinations (DM, HTN, DM+HTN, HTN + implanted stent, DM + implanted stent, DM + angina pectoris/arrhythmia)

Parametri	DM (n=52)	DM+HTA (n=139)	HTA (n=55)	HTA+stent (n=11)	DM+stent (n=19)	DM+AP/AF (n=29)	P
Gender (m/f), n(%)	31/21 (59.6/40.4)	64/75 (46.0/54.0)	16/39 (29.1/70.9)	6/5 (54.5/45.5)	15/4 (78.9/21.1)	11/18 (37.9/62.1)	19.7, 0.003
Age (years)	58 (46-66)	68 (62-72)^{aaa}	68 (62-74)^{aaa}	72 (66-79)^{aa}	68 (61-73)^{aa}	68 (64-74)^{aaa}	<0.001
BMI (kg/m^2)	26.7 (24.0-29.5)	28.6^{aa} (26.1-31.9)	27.5 (25.1-31.9)	28.2 (25.7-31.6)	27.8 (25.2-30.8)	29.5 (26.9-32.6)	0.080
Systolic pressure (mm Hg)	129 (125-132)	135 (130-140)^{aaa}	135 (130-145)^{aa}	130 (130-138)	130 ^{b,c} (120-135)	130 (120-145)	0.001
Diastolic pressure (mm Hg)	82 (80-85)	83 (80-86)	84 (78-86)	85 (75-88)	80 (70-85)	80 (75-85)	0.536
Smoking, no/yes, (%)	46/6 (18.6/10.3)	110/29 (44.5/50.0)	41/14 16.6/24.1)	10/1 (4.0/1.7)	16/3 (6.5/5.2)	24/5 (9.7/8.6)	4.9, 0.560
Sugar (mmol/L)	6.6 (5.5-8.8)	6.8 (5.6-8.4)	5.5 ^{aaa,bbb} (4.9-5.7)	4.9 ^{aa,bbb} (4.8-5.3)	6.7 ^{ccc,ddd} (6.4-7.3)	6.4 ^{ccc,ddd} (5.3-8.0)	<0.001
HbA1c (%)	7.1 (6.2-7.9)	6.5 (6.1-7.5)	5.8 ^{aaa,bbb} (5.7-5.9)	6.2 ^{c} (6.1-6.3)	6.4 ^{ccc} (6.2-8.0)	6.4 ^{cc} (5.9-7.0)	<0.001
Cholesterol, total (mmol/L)	5.07 (4.39-5.76)	5.09 (4.28-5.97)	5.72 ^{a,b} (4.92-6.12)	5.39 (4.65-5.97)	4.60 ^{c} (4.15-5.65)	4.88 ^{cc} (4.36-5.40)	0.047
LDL-C (mmol/L)	2.86 (2.30-3.72)	2.71 (2.15-3.53)	3.55 ^{aa,bbb} (2.88-3.90)	2.48 (2.34-3.31)	2.46 ^{cc} (1.96-2.73)	2.68 ^{cc} (2.03-3.18)	0.001
HDL-C (mmol/L)	1.25 (1.03-1.56)	1.25 (1.06-1.51)	1.42 ^{b} (1.16-1.66)	1.36 (1.28-1.90)	1.19 (1.05-1.39)	1.33 (1.19-1.68)	0.112
Triglycerides (mmol/L)	1.54 (1.06-2.38)	1.90 ^{a} (1.40-2.67)	1.53 ^{bbb} (1.21-1.87)	1.67 (1.15-2.50)	1.65 (1.18-3.33)	1.57 ^{bb} (1.05-2.10)	0.003
RPR index	0.051 (0.044-0.060)	0.049 (0.043-0.059)	0.055 ^{bb} (0.048-0.061)	0.062 ^{a,b} (0.046-0.067)	0.059 ^{a,bb} (0.055-0.066)	0.058 ^{b} (0.049-0.074)	<0.001
MPR index	0.038 (0.032-0.048)	0.038 (0.033-0.047)	0.041 (0.036-0.048)	0.045 (0.041-0.047)	0.046 ^{a,bb,c} (0.043-0.052)	0.039 (0.030-0.056)	0.008

A higher proportion of women was observed in the groups of patients with hypertension (HTN) and the combination of diabetes (DM) and hypertension, as well as in the group with diabetes and angina pectoris/AF. The youngest group consisted of patients diagnosed with diabetes, while the oldest group comprised patients with hypertension and an implanted stent. Patients with diabetes had the highest glucose and HbA1c concentrations. Patients with hypertension exhibited the highest total cholesterol and LDL-C levels.

Triglycerides were highest in the DM+HTN group, whereas HDL-C was lowest in the group of patients with DM and a stent, although this was not statistically significant. The same group had the highest NLR index, while the RPR index was highest in patients with HTN+stent, and the MPR index was highest in the group of patients with DM plus stent..

Table 2. Factor analysis in the group of patients with DM and/or various cardiovascular diseases

Factors	Parameters extracted into factors	loadings	Variability (%) Total: 45%
Age-hematological parameters related factor	Erythrocytes Hemoglobin Age (years)	0.836 0.784 -0.611	16
Metabolic parameter - renal function related factor	Uric acid Triglycerides Creatinine	0.720 0.679 0.516	15
A factor associated with blood pressure	Diastolic blood pressure Systolic blood pressure	0.814 0.801	14

Of all the measured parameters in this study, factor analysis produced three main factors. The first factor, named the "Age-Hematological Parameter Related Factor," consisted of the number of erythrocytes, hemoglobin concentration, and age (years), and accounted for 16% of the variability. The second factor, the "Metabolic-Renal Function Related Factor," included uric acid, creatinine, and triglycerides, explaining 15% of the variability. The third factor, the "Blood Pressure Related Factor," comprised systolic and diastolic blood pressure and accounted for 14% of the variability. The relationship between the factors extracted by factor analysis and the status of metabolic/cardiovascular disease was assessed using logistic regression analysis..

Table 3. Logistic regression analysis of the predictors extracted through factor analysis, expressed as factor scores (continuous variables), was performed for DM, CVD, or their combinations.

	Diabetes mellitus (DM) Number				**CVD**				**DM+ CVD**			
Factors	B (SE)	Wald koef.	OR (95% CI)	P	B (SE)	Wald koef.	OR (95% CI)	P	B (SE)	Wald koef.	OR (95% CI)	P
Age-hematological parameters related factor	1.015 (0.607)	2.80	feb.76 (0.840-9.06)	0.094	-1.18 (0.450)	6.94	0.306 (0.127-0.738)	0.008	0.657 (0.365)	3.23	1.93 (0.942-3.94)	0.072
Metabolic parameter - renal function related factor	-0.490 (0.669)	0.53	0.613 (0.165-2.274)	0.464	-0.464 (0.341)	1.85	0.629 (0.322-1.23)	0.173	0.570 (0.341)	2.80	1.77 (0.908-3.45)	0.094
A factor associated with blood pressure	-0.312 (0.440)	0.50	0.732 (0.309-1.73)	0.477	-0.012 (0.224)	0.003	0.988 (0.637-1.53)	0.958	0.091 (0.219)	0.173	01.t (0.713-1.68)	0.677

Platelet activation (MPR) and erythrocyte activation (RPR) indices were an important part of this study, aimed at detecting increased blood coagulability in patients using routine parameters obtained from a complete blood count. The distribution of values for these two novel parameters is shown in Figure 1, along with the distribution of the neutrophil-to-lymphocyte ratio (NLR)..

Figure 1. Distribution of RPR (A), MPR (B), and NLR (C) values in the group of patients with DM and CVD

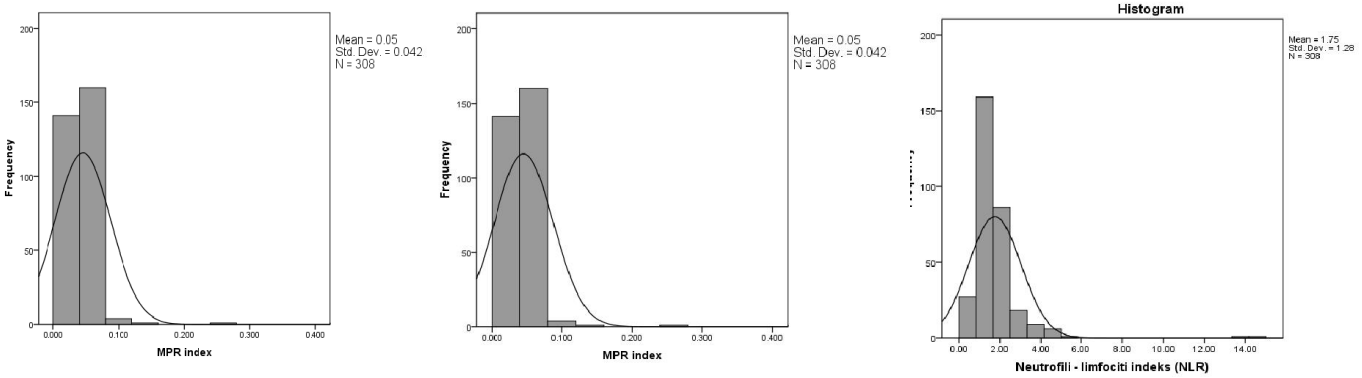

Table 4. Multiple linear regression analysis of predictors for MPR, RPR, and NLR values

Model for MPR	**B (95% CI)**	**SE**	**P**
Arrhythmia/AF	0.010 (0.002-0.017)	0.004	0.015
Stent	0.011 (0.002-0.020)	0.004	0.015
BMI (kg/m2)	-0.001 (-0.001-0.000)	0.000	0.011
Systolic blood pressure (mmHg)	0.000 (0.000-0.000)	0.000	0.064
Creatinine (μmol/L)	0.000 (0.000-0.000)	0.000	0.002
Model for RPR	**B (95% CI)**	**SE**	**P**
BMI (kg/m2)	-0.001 (-0.001-0.000)	0.000	0.005
Creatinine (μmol/L)	0.000 (0.000-0.000)	0.000	0.009
AST (U/L)	0.000 (0.000-0.001)	0.000	0.088
Model for NLR	B (95% CI)	SE	P
Hgb (g/L)	0.015 (0.002-0.028)	0.006	0.027

CI- confindence interval, SE – standard error

A multiple linear regression analysis was applied to identify models of significant predictors for the RPR and MPR indices. All measured parameters and clinical data were included in the analysis, and the results are presented in Table 4.

The MPR value was determined by a model consisting of the presence of arrhythmia (atrial fibrillation – AF), body mass index (BMI), systolic blood pressure (SBP), and creatinine levels (adjusted R^2 = 0.165). The RPR index was determined by a model including the presence of a coronary stent, BMI, creatinine concentration, and aspartate aminotransferase (AST) (adjusted R^2 = 0.145).

From the adjusted R^2 values, it can be concluded that the selected best model of predictors for MPR explains approximately 16.5% of the variability of this parameter, while the RPR parameter variability is explained by the best model at about 14.5%. The NLR value was determined by a model including only one parameter, hemoglobin (adjusted R^2 = 0.055), indicating that this model explains 5.5% of the variation in NLR..

DISCUSSION

The results of this study show significant differences in parameters such as BMI, glucose, HbA1c, blood pressure, and lipids among different groups of patients. These differences highlight the need for an individualized approach in the assessment and management of patients with cardiovascular diseases. The observed variations emphasize the importance of regular monitoring of these key parameters to timely identify patients at increased risk of complications.

A total of 311 participants were included in the study, with more than half (59.6%) being female. The average age of participants was 58 years. The sample was retrospectively selected through an analysis of patients' electronic health records who met specific criteria, with a consecutive sampling method. In the prospective part of the study, clinical and laboratory parameters were monitored during each patient visit.

Analysis of clinical and laboratory parameters in patients with cardiovascular and metabolic diseases revealed key biomarkers that provide valuable information about the patients' condition. BMI, glucose, HbA1c, total cholesterol, LDL-C, HDL-C, and triglycerides are essential for understanding the pathophysiology and risk associated with different health conditions. BMI is a reliable indicator of body mass relative to height and is often associated with the risk of developing cardiovascular and metabolic disorders [16].

Blood glucose levels and HbA1c are key indicators for diabetes management, reflecting glycemic control and variability, while total cholesterol and its fractions, such as LDL-C and HDL-C, assess the patient's lipid status [23]. The study by Artha et al. also examined the relationship between lipid profiles, lipid ratios, and glycemic control in patients with type 2 diabetes. It showed that higher levels of total cholesterol, low-density lipoprotein, triglycerides, and lipid ratios correlate with poorer glycemic control, as indicated by higher

HbA1c levels [23]. In contrast, the study by Kidwai et al. observed that high-density lipoprotein levels were lower in patients with poor glycemic control [24].

Accordingly, the LDL-C/HDL-C ratio has been identified as a significant risk factor for poor glycemic control, with a high ratio increasing the risk 38-fold. However, it is important to note that while this ratio is useful in theoretical assessment, in practice, the prognostic value of lipoproteins (Lp(a)), apolipoprotein A (apo A), apolipoprotein B (apo B), and the presence of small dense LDL particles may play a more significant role in risk assessment and cardiovascular disease management. These findings suggest that lipid profiles and ratios can serve as predictive markers for glycemic control, helping to manage cardiovascular risk in patients with diabetes. Additionally, regular monitoring of lipid levels alongside glycemic control is emphasized as crucial for reducing diabetes-related cardiovascular complications..

levated triglyceride levels can be indicators of metabolic syndrome and are associated with an increased risk of coronary artery disease (CAD) [23]. Analysis of basic clinical and biochemical data revealed that patients with diabetes (DM) had significantly higher BMI, glucose, HbA1c, and HDL-C levels compared to the upper limit of reference values, while their diastolic blood pressure was significantly lower than this threshold. Accordingly, in our study, BMI, glucose, and HbA1c showed significant variations among patients with DM, hypertension (HTA), and their combinations. These parameters are key indicators of metabolic health, and their control is crucial for reducing the risk of complications. Differences in systolic and diastolic blood pressure among the groups further highlight their contribution to cardiovascular risk, especially in patients with HTA and implanted stents.

The study by Britton et al. investigated the correlation between hemoglobin A1c (HbA1c), body mass index (BMI), and the risk of developing hypertension (HTA). The aim was to determine whether there is a prospective association between baseline HbA1c values and the incidence of HTA over time, considering the role of body weight. This study is particularly important, as elevated HbA1c may indicate poor blood sugar control, a risk factor for developing hypertension, while BMI is a well-known indicator of body mass that can also influence cardiovascular health. The authors' conclusions are consistent with the findings of our study [26]. Britton's study emphasizes the importance of BMI, glucose levels, and HbA1c as key indicators of metabolic health. Elevated BMI may indicate overweight or obesity, factors that can increase hypertension risk due to added strain on the heart and blood vessels. High HbA1c levels suggest chronic hyperglycemia, which can lead to vascular damage and inflammatory processes, further increasing cardiovascular risk. These findings are supported by other published studies [27,28], reinforcing our hypothesis regarding the link between these variables and hypertension risk.

Our findings align with previous research showing that elevated HbA1c and BMI are associated with higher hypertension risk. For example, some studies indicate that even mildly elevated HbA1c levels may be indicative of increased HTA risk, while other evidence suggests that interventions aimed at reducing BMI can lower hypertension incidence in patients with metabolic syndrome. These results suggest that weight management and glycemic control may be key elements in preventing hypertension, which is consistent with our study's findings.

Furthermore, an Israeli study from 2021 [29] provides additional evidence that elevated HbA1c and BMI levels significantly contribute to the development of hypertension. These results underscore the need for regular monitoring of metabolic factors such as HbA1c and BMI, especially in patients at increased cardiovascular risk. Taken together, it is clear that HbA1c and BMI are key health indicators that can significantly influence hypertension risk.

The results of that study also showed that HbA1c variability is an independent risk factor for diabetes-related complications. Approximately 22% of participants had high HbA1c variability, which was associated with a BMI of 30 or higher. This suggests that patients with higher BMI often have less stable blood sugar levels, increasing the risk of complications such as cardiovascular disease and neuropathy.

Additionally, patients with high HbA1c variability had more frequent visits to diabetes clinics, indicating a more complex disease profile requiring intensive monitoring and treatment. These patients often used insulin and ACE inhibitors, and their age as well as the presence of ischemic heart disease were also significant factors. The association between high HbA1c variability, younger age, and high BMI suggests that risk factors for complications often occur together, creating a more complex pattern of diabetes management.

These findings indicate that HbA1c variability can serve as a marker of disease complexity and lifestyle, highlighting the importance of monitoring HbA1c fluctuations. This study supports the observations from our research on the significance of BMI, glucose, and HbA1c as key indicators of metabolic health. It also emphasizes their role in reducing the risk of complications in patients with diabetes and hypertension. In light of these findings, it is important to consider the impact of HbA1c variability on clinical practice. Monitoring and analyzing these fluctuations can help clinicians better understand disease dynamics and identify patients at higher risk of developing serious complications. Implementing strategies for weight management and glycemic control may be crucial in preventing hypertension and other cardiovascular problems, further reinforcing the conclusions of our study..

Indices such as MPR, RPR, and NLR have proven useful in assessing procoagulant activity and inflammatory status, providing important insights into patients' health. These parameters can serve as early indicators of increased risk in patients with complex comorbidities, enabling timely intervention and tailored therapeutic strategies.

MPR, the monocyte-to-platelet ratio, can indicate changes in the immune and coagulation systems. RPR, the reticulocyte-to-platelet ratio, helps assess the regenerative capacity of the bone marrow and coagulation activity. NLR, the neutrophil-to-lymphocyte ratio, is a simple yet effective marker of inflammation and stress. By using these indices, clinicians can better understand and manage patient risks, potentially improving treatment outcomes and adjusting therapeutic plans according to individual patient needs.

Our study results highlight that MPR values were highest in patients with diabetes (DM) and an implanted stent. This index may be particularly useful in assessing thrombotic risk, as it indicates platelet activation, which is often associated with increased blood coagulability. In our research, the presence of a stent and factors such as atrial fibrillation (AF) and other arrhythmias were identified as significant predictors of MPR values, suggesting a higher predisposition to thrombotic events in these patients.

Furthermore, RPR values were highest in patients with hypertension (HTA) and an implanted stent. This index may reflect changes in the hematopoietic system and can be useful in evaluating coagulation status. Our study indicated that the presence of a stent, BMI, and creatinine levels significantly influenced RPR values, which is important for identifying patients potentially at increased risk of coagulation-related complications.

Although less dominant in our model, NLR was recognized as a significant marker of inflammation. Our findings emphasize that NLR can serve as a useful tool for the early identification of patients at higher risk of complications, allowing clinicians to adjust therapeutic strategies and improve outcomes. When analyzed alongside other clinical and laboratory parameters, NLR contributes to more precise monitoring and management of patients with complex comorbidities.

The literature also underscores the importance of other inflammatory markers, such as hsCRP (high-sensitivity C-reactive protein), commonly used to assess inflammatory status and cardiovascular risk. Elevated hsCRP levels have been associated with increased complication risk in patients with chronic conditions, including diabetes and hypertension [37,38]. Compared to NLR, hsCRP offers the advantage of high sensitivity for detecting low-grade inflammation. While NLR provides insight into the balance between neutrophils and lymphocytes, reflecting general immune status, hsCRP directly measures inflammation levels in the body. The complementary nature of NLR and hsCRP enhances understanding of inflammatory processes in patients with complex

comorbidities. Studies investigating inflammatory markers, including NLR and hsCRP, have shown that these indices can serve as early indicators of increased risk in such patients [38]. Considering their complementary roles, clinicians may consider using both markers in routine risk assessment, enabling timely intervention and optimized therapeutic approaches.

For example, Thurston et al. found that higher NLR values often reflect an increased inflammatory response, which is associated with cardiovascular risks [30]. This study, like ours, suggests that these markers can serve as early risk indicators in patients with complex comorbidities, providing important information on health status and potential cardiovascular complications. Additionally, Li et al., in a study published in early November 2024, highlighted the exceptional value of the neutrophil-to-lymphocyte ratio in assessing cardiovascular risk [31]. Analyzing data from 2,239 participants with cardiovascular disease (CVD), they found that higher NLR values correlated with increased risk of all-cause and cardiovascular mortality. Using data from the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2018, the study demonstrated that higher NLR independently predicted increased mortality risk, even after adjusting for demographic and clinical factors. Each one-unit increase in NLR was associated with a 15% higher risk of all-cause mortality (HR: 1.15; 95% CI: 1.11–1.19) and a 14% higher risk of cardiovascular mortality (HR: 1.14; 95% CI: 1.08–1.20) in a model including all relevant variables. Restricted cubic spline analysis indicated a nonlinear relationship between NLR and all-cause mortality ($p<0.05$ for nonlinearity), suggesting that increasing NLR may have a more complex relationship with mortality risk, particularly at higher levels. These findings support our conclusion that NLR, like other inflammatory indices such as MPR and RPR, can be effective indicators for risk stratification and prognosis assessment in patients with CVD. The study emphasizes NLR's potential as a cost-effective clinical marker.

Factor analysis identified the "age-hematologic factor" and "metabolic-renal factor" as significant variables related to risk. These factors consolidate variability among clinical and biochemical parameters, providing deeper insight into the complex interactions contributing to cardiovascular risk. Notably, the blood pressure-related factor showed a significant contribution to overall risk assessment, underscoring the need for its management in clinical practice.

Our study identified the "age-hematologic factor," which includes red blood cell count, hemoglobin concentration, and age. This factor accounted for 16% of variability and demonstrates how age and hematologic characteristics may be associated with increased cardiovascular risk. The association of age with hematologic parameters, such as anemia, is often correlated with higher CVD risk, making our analysis relevant for assessing overall patient health.

A relevant study addressing age and hematologic factors in the context of cardiovascular risk is by Truslowa et al. Like our research, it investigated the use of hematologic markers from complete blood counts in developing predictive models for cardiovascular events [32]. That study used Cox proportional hazards models to predict outcomes such as myocardial infarction, ischemic stroke, heart failure hospitalization, revascularization, and all-cause mortality. While the modeling methodology differs from ours, the results showed that models including hematologic indices provide better predictions than models using only demographic data and diagnostic codes. Specifically, models performed best in predicting heart failure and all-cause mortality, with concordance indices ranging from 0.60 to 0.80. Consequently, Truslowa et al. highlight the potential of using hematologic markers, such as red blood cell count and hemoglobin concentration, in assessing cardiovascular risk, supporting our observations regarding the "age-hematologic factor.".

The blood pressure factor consists of systolic and diastolic blood pressure and accounts for 14% of variability. Our study highlights the importance of this factor in overall risk assessment. Hypertension (HTA) is a well-known risk factor for cardiovascular disease (CVD), and blood pressure control is essential to reduce the risk of heart attacks and strokes. These results are consistent with previously published studies examining the correlation

between HTA and CVD [28,33]. The discussion of this factor emphasizes its practical application in clinical settings, where monitoring and managing blood pressure is crucial for preventing cardiovascular events.

Limitations of this study include its retrospective design and a sample restricted to a single healthcare institution. Future research could include longitudinal studies to track long-term outcomes, as well as investigation of additional biomarkers that may improve risk assessment in similar patient populations..

CONCLUSION

The results of this study indicate that inflammatory-hemostatic indices, such as MPR and RPR, together with clinical factors, serve as useful tools for risk assessment in patients with cardiovascular diseases. The highest MPR values were observed in patients with diabetes and an implanted stent, while the highest RPR values were found in patients with hypertension and a stent, suggesting a pronounced inflammatory and procoagulant status in these subgroups.

Factor analysis further confirmed the significance of the age-hematological cluster as the sole independent predictor of the presence of cardiovascular disease. These findings underscore the importance of integrating simple, routinely available hematological markers with clinical data to enable early identification of high-risk patients.

Incorporating these parameters into daily clinical practice may enhance diagnostics, allow for personalized therapy, and contribute to more effective primary and secondary prevention of cardiovascular diseases..

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LYME DISEASE

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Summary: Lyme disease (LD) is a multisystem infectious disease caused by bacteria of the Borrelia genus, most commonly Borrelia burgdorferi, and transmitted through the bite of an infected tick (Ixodes ricinus). Rodents (mice, rats) are the primary reservoirs of the bacteria. Transmission occurs most frequently between May and August in temperate climate zones, although the disease can appear outside this period depending on climatic conditions and tick activity. In its early stage, Lyme disease most commonly manifests as a characteristic skin lesion known as erythema migrans, which begins as a red macule or papule and can expand up to 50 cm in diameter, with central clearing and clearly or slightly defined borders. Other symptoms may include headache, fever, fatigue, muscle and joint pain, which can lead to misdiagnosis or delayed treatment. If left untreated, the disease can cause serious systemic complications, including neurological disorders (e.g., meningitis, neuropathies), cardiac problems (atrioventricular block), and arthritis, most often affecting the knees. The late stages of the disease can last for months or years, but with adequate antibiotic therapy, symptoms can often be reduced. Diagnosis is based on the clinical presentation and serological tests such as ELISA and Western blot, which detect the presence of antibodies to the bacteria. It is important to note that serological tests may be negative in the early phase of infection, as antibodies have not yet developed. PCR tests can confirm the presence of bacteria through direct examination of blood, cerebrospinal fluid, or tissue samples. However, in routine diagnosis, the presence of erythema migrans as a clinical finding is considered a sufficient reason to initiate therapy. Prevention focuses on reducing contact with ticks, including wearing appropriate protective clothing, using repellents, and treating clothing with permethrin. Preventive measures also include mowing and maintaining grassy areas, as well as controlling rodent and tick populations in places where people live or spend time.

Keywords: Lyme disease, Borrelia burgdorferi, Ixodes ricinus, vector-borne infection, erythema migrans, bacterial transmission, diagnosis, serological tests, prevention, complications, arthritis, neurological disorders, ticks, and rodents

DEFINITION AND EPIDEMIOLOGY OF LYME DISEASE

Lyme disease (LD), or MORBUS LYME, is a chronic multisystem infectious disease in humans, transmitted through the bite of an infected hard tick, Ixodes ricinus, carrying one of the bacteria such as Borrelia burgdorferi (Bb) (Figure 1), and less commonly other Borrelia species: Borrelia garinii (Bg) and Borrelia afzelii (Ba).

It is a multisystem disease that can affect the skin, joints, heart, and nervous system. Lyme disease is particularly known for its frequent manifestation as the characteristic “erythema migrans”, a bull’s-eye-shaped skin rash [1].

Figure 1. *Borrelia burgdorferi* magnified 400 x

Source: https://upload.wikimedia.org/wikipedia/commons/f/f3/Borrelia_burgdorferi_%28CDC-PHIL_-6631%29_lores.jpg

When discussing the developmental stages of ticks, the starting point is the egg, which the female (adult) lays in early spring. From these eggs, larvae hatch in early summer. The larva takes its first blood meal from the nearest available animal—most often micromammals (forest and field rodents, hedgehogs, and others). Humans can also occasionally be bitten by larvae, although this occurs much less frequently compared to other developmental stages of the tick.

After feeding, the larva molts (matures) into a nymph, which then takes a second blood meal from the nearest host. For nymphs, these hosts typically include hares, birds, deer, and occasionally humans who spend time in tick habitats. Borrelia is transmitted through tick saliva during feeding, usually after 48 hours or longer.

There are three theories regarding the transmission of Borrelia burgdorferi (Bb) from the tick to the next host, with two being most widely accepted. The first suggests that during the tick's intense blood-feeding phase, once it becomes engorged, it regurgitates part of its intestinal contents (Bb resides in the tick's midgut). The second, less common but still recognized in the literature [1,2], proposes that Bb migrates from the midgut to the salivary glands. This theory implies a transmission mechanism similar to that of mosquitoes transmitting Plasmodium. Advocates of this theory often recommend prophylactic antibiotic treatment after every tick bite, which is incorrect.

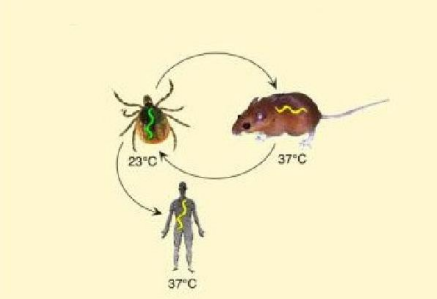
Experimental studies on the transmission of Borrelia from infected ticks to mice have shown that infection rarely occurs within the first 24 hours of tick attachment. The likelihood of infection increases with the tick's duration of attachment—particularly after 48 hours, and especially after 72 hours. Therefore, information about the tick's attachment time (less than 24 hours) is extremely important for the prevention of Lyme disease. Prompt and proper removal of the tick within the first few hours can be crucial, especially if the tick is infected with Bb [3].

In Lyme disease, the reservoir represents the ecological niche—the place within the host (tick, small rodent, etc.) where the pathogen lives, persists as a species, and/or reproduces, usually without harming its host.

In the case of Lyme disease, the tick can serve both as a reservoir of Bb and as the source of infection (the one that directly transmits Bb to a new host). Once infected, a tick can transmit Bb throughout all its developmental stages—from larva to adult—and even transovarially (from female to offspring). The vector, or carrier of the pathogen, is Ixodes ricinus (Europe), Ixodes pacificus and Ixodes scapularis (America), Ixodes persulcatus (Asia), etc., while the causative agent belongs to the Borrelia burgdorferi genospecies [4,5].

The term "Borrelia cycle" is translated as either the life cycle of Borrelia or the enzootic cycle of Borrelia in English. It refers to the complex life cycle of the Lyme disease bacterium (Borrelia burgdorferi), which alternates between tick vectors and vertebrate hosts. This enzootic cycle involves the transmission of the bacterium from an infected tick to a host, and potentially back to another tick (Figure 2) [6].

Figure 2. life cycle (transmission cycle) of Borrelia, which alternates between the tick vector and the vertebrate host.
Source: https://upload.wikimedia.org/wikipedia/commons/0/08/Borrelia_cycle.jpg



CLINICAL ASPECTS OF LYME DISEASE

Lyme disease is the most common vector-borne infectious disease in Europe and North America. LD is typically a seasonal illness, occurring during periods of tick activity—from early spring and the first warm days (nymphal

stage), throughout June (larval and nymphal stages), and up to the late autumn months (adult stage). During the rest of the year, when tick activity ceases, Lyme disease does not occur.

When searching for a diagnosis in patients presenting with symptoms suggestive of LD, serological testing is most commonly used to raise clinical suspicion. The incubation period ranges from 3 to 30 days, from the tick bite to the appearance of signs and symptoms of Lyme disease. It is important to note that not every erythema at the site of a tick bite is Erythema migrans (EM). EM occurs in 60–80% of cases and is often accompanied by flu-like symptoms.

At the site of the tick bite, within 5–7 days or longer, a characteristic skin lesion may appear—Erythema migrans—which begins as a macule or papule and can enlarge to as much as 50 cm in diameter. EM presents as redness expanding from the bite site toward the periphery in the form of irregular concentric rings with serrated, more intensely red edges. The redness is flat, warm to the touch (like surrounding skin), and does not cause pain or itching [7,8,9].

This characteristic skin lesion—EM—is a hallmark sign of Lyme disease. It differs from other skin rashes because it lacks tumor (swelling) and dolor (pain), and the calor (warmth) is the same as in surrounding skin [10]. Alongside the lesion (EM), early symptoms—often flu-like—may include headache, mild fever (rare), chills, shivering (rare), muscle and joint pain, lymphadenopathy, and fatigue, which is profound, persistent, and unrelated to physical activity [10,11]. Symptoms typically last around four weeks [11].

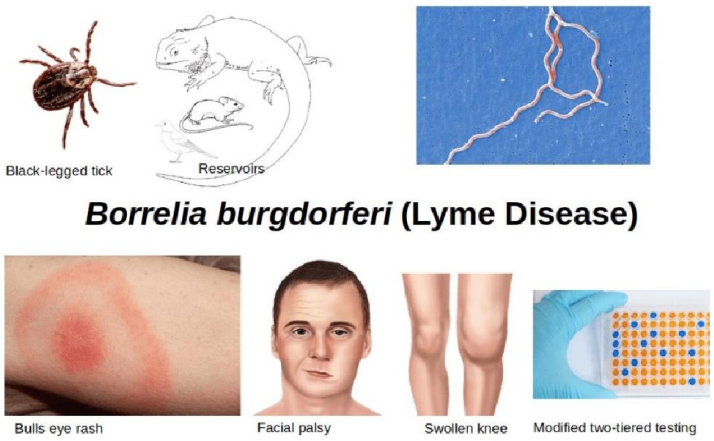
In untreated patients, after several weeks, hematogenous dissemination can occur, leading to systemic manifestations such as fatigue, myalgia, and skin, cardiac, and neurological disorders [10,11]. Arthritis develops in about 60% of patients, usually monoarticular or oligoarticular, predominantly affecting the knee joint—a sign of late-stage LD (third stage) [10,12].

Neurological manifestations occur in about 10–20% of patients, most commonly facial nerve palsy. This belongs to the secondary stage and is less common in Europe. The Centers for Disease Control and Prevention (CDC) identifies this symptom as frequent in the United States [12].

The second stage may also include carditis, occurring in approximately 8% of untreated, infected individuals, presenting with palpitations and atrioventricular (AV) conduction abnormalities, as well as electrocardiographic changes in the S-T segment and T wave [10,12].

The late stage, developing months or even years after untreated Lyme disease, leads to polyarthritis and chronic skin lesions with discoloration, known as acrodermatitis chronica atrophicans [11,13].

Figure 3. Borrelia Burgdorferi - Lyme disease
Source: https://i0.wp.com/microbeonline.com/wp-content/uploads/2021/05/Borrelia-Burgdorferi-Lyme-Disease-min.png?ssl=1

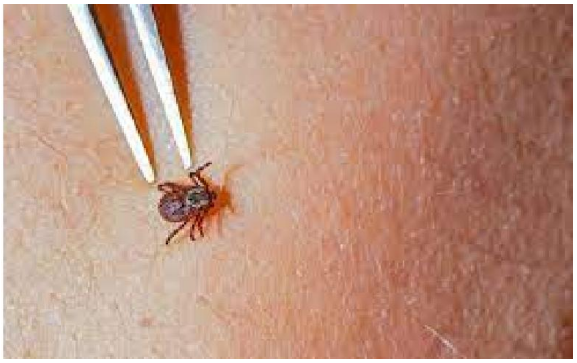


Prolonged attachment of a tick to the skin increases the likelihood of transmitting Borrelia burgdorferi. Therefore, timely removal of the tick is crucial for reducing the risk of infection. The longer the tick remains attached, the higher the probability of pathogen transmission. For this reason, prompt and proper tick removal is one of the most important steps in preventing the clinical manifestation of Lyme disease [14,15].

If a tick is observed on the body, it is recommended to remove it as soon as possible [16,17]. Ideally, this should be done in a healthcare facility, where a physician can assess the risk of infection and determine further management. If immediate professional removal is not possible, the tick can be removed independently using fine-tipped tweezers. The tweezers should grasp the tick as close to the skin as possible, near its head, and pull it out slowly, steadily, and evenly without sudden movements (Figure 4).

After removal, the bite site should be disinfected with alcohol or iodine [18]. Regardless of successful removal, it is recommended to see a physician promptly to evaluate the risk, monitor for potential symptoms, and decide whether further diagnostic or prophylactic measures are needed [17,19]. The key is not only proper tick removal but also monitoring one's health and seeking medical attention, as infection can occur even after the tick has been removed [16,17].

Figure 4. Removing ticks with tweezers
Source: https://www.bbc.com/serbian/lat/svet-69247310



Routine testing of ticks themselves for the presence of Borrelia burgdorferi or other pathogens is not recommended for clinical purposes, as a positive result does not confirm that infection has been transmitted, nor does it determine therapeutic management. The main criteria for deciding on prophylactic treatment include: the tick species (Ixodes), endemic region, duration of attachment (>36 hours), and time since removal (<72 hours)—as recommended by the IDSA/AAN/ACR Lyme disease guidelines (2020) [20].

LABORATORY DIAGNOSIS OF LYME DISEASE

The laboratory diagnosis of Lyme disease involves a combination of methods applied according to the stage of the disease and its clinical presentation [21,22]. The most accessible and widely used diagnostic approach is serological testing of blood samples (ELISA, confirmed by Western blot) [21,23]. In cases where neuroborreliosis is suspected, both serological testing and PCR analysis of cerebrospinal fluid (CSF) are performed [22,24].

The PCR method enables the direct detection of Borrelia burgdorferi DNA in blood, CSF, or specific tissue samples; however, a negative result does not exclude infection. Although removed ticks can also be tested by PCR for pathogen detection, such testing has epidemiological significance only and does not guide therapeutic decisions [21,25].

Proper interpretation of laboratory findings requires integration of test results with the clinical picture and epidemiological factors, since serological tests may yield false-positive or false-negative results, particularly in the early stages of the disease.

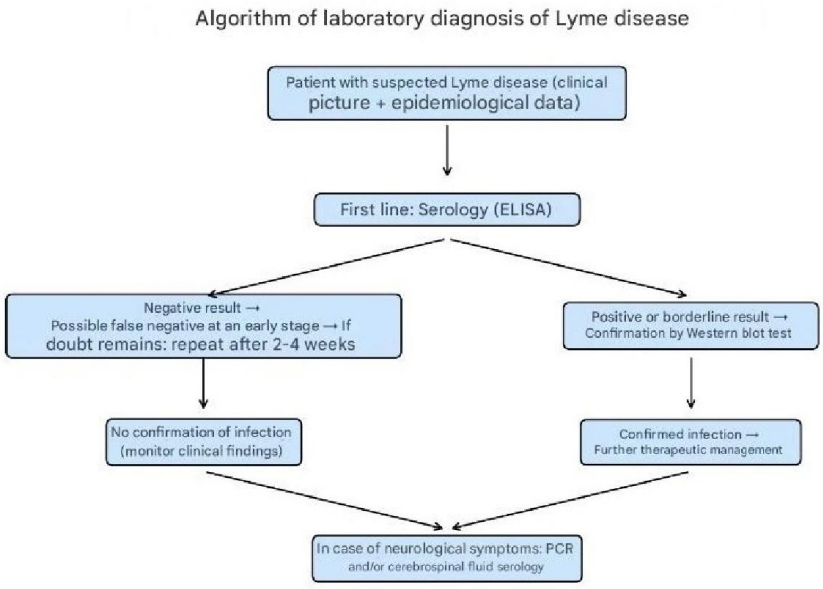


Table 1. Laboratory diagnostics

Sample	Method	Advantages	Limitations / Notes
Blood	Serology (ELISA → Western blot)	The most affordable method, widely available	Antibodies appear slowly (3–6 weeks); false positive and negative results can be >20%; requires clinical correlation
Blood	PCR	The possibility of DNA detection in the early stages of the disease	A negative result does not rule out infection; sensitivity varies in different stages of the disease
CSF	Serology / PCR	Useful in suspected neuroborreliosis	Invasive procedure; results depend on the stage of the disease
Synovial fluid / tissue	PCR	Specifically for arthritis or local infections	It is used only in selected cases; laboratory required process
Tick	PCR	Determination of the presence of Borrelia burgdorferi (epidemiological significance)	It is not used for clinical diagnostics; the finding does not determine the therapy

Ticks are not used for serological diagnosis in clinical practice. Their testing serves exclusively epidemiological purposes or research on the distribution of pathogens. Serological tests of blood and cerebrospinal fluid remain the cornerstone of routine laboratory diagnosis of Lyme disease.

Serological testing is the most accessible diagnostic approach (performed by almost all public health institutes) and represents the first step in the serological diagnosis of Lyme disease. However, these tests are neither highly specific nor highly sensitive, yielding more than 20% false-positive and false-negative results. It is also important to emphasize that antibodies to Borrelia burgdorferi develop slowly, and blood sampling should not be performed before the end of the third or fourth week after the onset of symptoms. Therefore, caution is required when interpreting serological results in Lyme disease.

The main serological tests used for diagnosis are ELISA and immunofluorescence assays (IFA). The ELISA test (Figure 5) for Borrelia burgdorferi identifies the presence of IgM and IgG antibodies, indicating whether it is an acute infection (IgM) or a past infection (IgG)—although it is important to note that the presence of IgG antibodies in Lyme disease does not always confirm a past infection, as it might in other diseases [26,15].

IgM antibodies usually appear 2–4 weeks after the onset of the erythema migrans lesion but are not always detectable at sufficient levels for serological identification and typically disappear after 4–6 months. In some cases, IgM antibodies may persist for several months after initial detection. IgG antibodies typically appear 8–12 weeks after the onset of illness and reach their peak within 4–6 months.

In the serological diagnosis of Lyme disease, the initial tests include ELISA, EIA (enzyme immunoassay), or IFA (immunofluorescence antibody assay). Negative results in the early phase of the disease do not exclude the diagnosis, as antibodies may still be insufficiently developed—particularly if antibiotic therapy was initiated early or if erythema migrans is still present. Positive or borderline results should be confirmed using the Western immunoblot test (Figure 6).

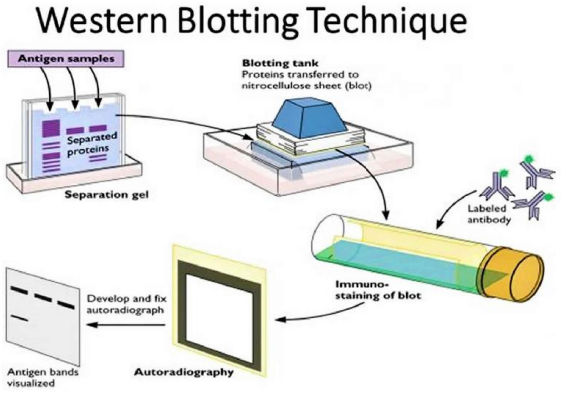
If serological results are negative, but clinical symptoms of Lyme disease persist, it is recommended to repeat testing after 2–4 weeks [27,28].

Figure 5. How to choose the right propeller kit

Source: https://www.bmgrp.com/how-to-choose-the-right-elisa-kit/

Figure 6. Western blotting technique, used to detect specific proteins in samples

Source: https://healthjade.net/western-blot/



In addition to these tests, PCR is less commonly used in diagnostics and is recommended for testing the tick itself for the presence of Lyme disease pathogens (primarily for research purposes, not routine diagnostics). PCR is also the only method used in everyday diagnostics, apart from the cultivation of Borrelia on BSK II medium, which is performed only in research settings.

False-positive serological results for Lyme disease can occur in patients with syphilis. Early diagnosis and timely administration of antimicrobial therapy play a key role in preventing cardiac, neurological, and musculoskeletal complications. It is important to note that antibiotics in the initial stage of Lyme disease do not prevent the development of symptoms in later stages but serve as prophylactic treatment to reduce the risk of disease progression..

TREATMENT OF LYME DISEASE

In the treatment of early Lyme disease, oral antibiotics such as amoxicillin, doxycycline, or cefuroxime are used. The choice of antibiotic and duration of therapy depend on the patient's age and the clinical stage of the disease. Doxycycline is generally avoided in children under 8 years due to potential effects on teeth and bones, while amoxicillin is preferred in pregnant women.

For patients with recurrent arthritis or involvement of the central or peripheral nervous system, parenteral therapy with intravenous antibiotics—most commonly ceftriaxone, cefotaxime, or penicillin G—is administered. IV therapy is reserved for severe or chronic forms of the disease, with dose, duration, and patient age being crucial factors for treatment effectiveness and complication prevention.

Lyme disease therapy is guided by the clinical form, disease severity, and patient age. In early localized forms, oral antibiotics—doxycycline, amoxicillin, or cefuroxime—are prescribed, with restrictions for children under 8 years and pregnant women. Treatment duration ranges from 10 to 21 days, depending on the antibiotic and clinical presentation. For more severe or chronic forms, including neuroborreliosis and recurrent arthritis, parenteral therapy with intravenous ceftriaxone, cefotaxime, or penicillin G is used, typically for 14–28 days. Treatment efficacy depends on timely administration, appropriate dosage, therapy duration, and patient age. [29,30].

Table 2. Lyme Disease Therapy: Antibiotics, Dosages, and Duration

Form of Lyme disease	Antibiotic	Dose (adult)	Duration of therapy	Notes / age
Early localized (erythema migrans)	Doxycycline	100 mg orally 2× daily	10–21 days	Not recommended for children <8 years; not for pregnant women
	Amoxicillin	500 mg orally 3× daily	14–21 days	Suitable for children and pregnant women
	Cefuroxime axetil	500 mg orally 2× daily	14–21 days	Alternative for children and adults
Early disseminated / neuroborreliosis	Ceftriaxone i.v.	2 g daily	14–28 days	Application in severe and chronic forms
	Cefotaxime i.v.	2 g every 8 hours	14–28 days	-
	Penicillin G i.v.	18–24 million IU per day in 4–6 doses	14–28 days	-
Recurrent arthritis / central and peripheral nervous system	Ceftriaxone i.v.	2 g daily	14–28 days	More severe forms of the disease, the age of the patient affects the dose
	Cefotaxime i.v.	2 g every 8 h	14–28 days	-
	Penicillin G i.v.	18–24 miliona IU per day in 4–6 doses	14–28 days	-

Notes: Doxycycline is not used in children under 8 years of age and in pregnant women due to the risk to teeth and bones. Oral therapy is applied in early localized forms. I.V. therapy is used in severe, disseminated, or chronic forms, in cases of neuroborreliosis and recurrent arthritis. The duration of therapy can be adjusted according to the patient's clinical response..

PREVENTION

Control of ticks in areas frequently visited by people (parks, forested parks, recreational areas) represents a fundamental measure for preventing tick bites and, consequently, reducing the risk of Lyme disease transmission. Preventive activities can be divided into ecological control measures, personal protective measures, and public health interventions:

Ecological control measures include the application of appropriate acaricides on limited areas with high tick populations, mowing and maintenance of grassy areas—especially in places used for recreation and play—removal of leaves, low vegetation, and branches in parks and yards to reduce suitable tick habitats, control of rodent populations that are natural reservoirs of Borrelia burgdorferi, and minimizing human-rodent contact.

Personal protective measures involve wearing appropriate clothing when in nature: long sleeves, long pants tucked into socks, closed shoes, and light-colored clothing to facilitate tick detection; using repellents based on DEET, icaridin, or permethrin (on clothing), especially for individuals spending extended time outdoors in endemic areas; and performing a full-body tick check after outdoor activities, including hair, skin folds, and areas where ticks commonly attach.

Public health interventions include educating the population about the risks of tick bites, protection methods, and the importance of early tick removal; organizing tick control campaigns in public areas during peak activity seasons (spring and summer); monitoring and surveillance of tick populations in endemic regions; and risk mapping for the local population. [31-33].

CONCLUSION

Lyme disease represents a significant public health problem in endemic areas of Europe and North America. Prevention is based on reducing contact with ticks, wearing protective clothing, using repellents, and implementing ecological measures to control tick and rodent populations. Diagnosis is primarily clinical, supported by serological testing, while molecular methods serve as supplementary diagnostic tools. Timely and appropriate antibiotic therapy in the early stage of the disease is crucial for preventing systemic complications. Educating the public and healthcare personnel, as well as proper tick removal, are the most effective strategies for controlling and preventing Lyme disease.

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TIMOK MEDICAL GAZETTE

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DIAGNOSIS AND TREATMENT OF PELVIC VENOUS CONGESTION IN WOMEN

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Abstract: Introduction: Pelvic venous congestion syndrome (PVCS) can be defined as a disorder of the pelvic venous system, i.e. the presence of pelvic venous insufficiency (PVI) that manifests itself with a wide range of symptoms and signs. PVCS affects women of reproductive age and often presents with chronic pelvic pain. Other symptoms include pelvic heaviness, dyspareunia, dysmenorrhea, low back pain, frequent and urgent urination, and signs of dilated vulvar, perineal, gluteal superficial veins or varicose veins of the lower extremities and hemorrhoids. PVCS can be caused by a combination of several factors: genetic predisposition, anatomical abnormalities, hormonal factors, dysfunctional valves, obstruction of venous flow, and damage to the vein walls. Diagnostics: The diagnosis of PVCS remains a major challenge, given the lack of universally accepted criteria in diagnostic imaging modalities. The following imaging methods can be used for diagnosis: ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI) as non-invasive methods and venography (VG) as an invasive method. Pelvic ultrasound is usually the first-line method. Transcatheter venography remains the gold standard for the diagnosis of PVCS. Treatment: Conservative medical treatment for PVCS is limited due to the lack of data on long-term efficacy. Compression is one of the therapeutic options for conservative treatment. Embolization is recommended for the treatment of PVCS. Clinical improvement after embolization ranges from 47 to 100% in different studies, but future randomized trials are needed to determine clear protocols for the management of embolization. Conclusion: PVCS is a common cause of chronic pelvic pain in women, but due to lack of knowledge, it is often not recognized and remains undiagnosed. In the absence of other causes of PVCS, PVI should also be considered. Additional education of gynecologists for the use of ultrasound in the diagnosis of PVCS is needed, given that ultrasound is the initial imaging method.

Key words: pelvic congestive syndrome, pelvic venous insufficiency, chronic pelvic pain, embolization.

INTRODUCTION

Pelvic venous congestive syndrome (PVCS) can be defined as a disorder of the pelvic venous system, namely the presence of pelvic venous insufficiency (PVI), which manifests through a wide spectrum of symptoms and signs. By definition, the primary cause of this syndrome is pelvic venous insufficiency, indicated by dilation and dysfunction of the ovarian veins, internal iliac veins with their tributaries, as well as venous plexuses.

PVCS affects women of reproductive age and is often associated with chronic pelvic pain lasting at least six months [1]. Pain is frequently described as a typical symptom of PVCS, characterized as chronic, dull, unilateral or bilateral [2]. Other symptoms include a feeling of pelvic heaviness, dyspareunia,

dysmenorrhea, lumbar pain, frequent and urgent urination, and signs of dilated vulvar, perineal, gluteal superficial veins or varicosities of the lower extremities and hemorrhoids [1]. Chronic pelvic pain is not necessary for diagnosis, as in many patients the predominant symptom may be atypical superficial varicosities. In some women, superficial varicose veins may be the only sign of PVI. The prevalence of vulvar varicosities in patients with PVCS ranges from 24–40%. Up to 80% of patients with pelvic venous dilation may exhibit varying degrees of associated venous insufficiency of the lower extremities [2].

The complexity of the problem lies in the fact that different symptoms can occur at the same degree of PVI, just as the same symptoms may appear at different degrees of PVI. Chronic pelvic pain in women can arise from various causes, including endometriosis, adhesions, fibroids, adenomyosis, genital organ prolapse, malignancies, and many other causes; very often, in the absence of an explanation for the pain, chronic pain is attributed to psychosomatic disorders.

Due to the nonspecific symptomatology, PVCS is often unrecognized and underdiagnosed. Factors that exacerbate pelvic pain, as described in the literature, include prolonged periods of standing, walking, or sitting, as well as factors that increase intra-abdominal pressure, such as lifting and pregnancy. Pain generally worsens during the day, as well as before and in the first days of menstruation, and decreases when lying down.

Pain also increases during and after sexual intercourse. Osman et al. reported that dyspareunia due to endometriosis is typically associated with deep penetration, whereas pain caused by PVCS usually worsens with sexual activity, producing a pulsating pain after intercourse [3].

Urinary symptoms may occur in PVCS due to perivesical varicosities, such as bladder irritability, urgency, or dysuria. Other manifestations of PVCS can include rectal discomfort, vulvar swelling, vaginal discharge, persistent genital arousal, and nonspecific gastrointestinal symptoms such as bloating and nausea. Chronic pelvic pain and these additional symptoms negatively affect patients' quality of life, leading to a significantly higher incidence of depression, anxiety, and generalized lethargy in this group. [1].

Anatomy

The pelvic venous system is responsible for returning venous blood from the walls and organs of the pelvis back to the central circulation. The external iliac vein (EIV) primarily drains the lower extremities, whereas the internal iliac vein (IIV) drains the pelvic organs, pelvic walls, gluteal region, and perineum. All veins from the pelvis and lower extremities generally converge into the inferior vena cava (IVC) and proceed to the right atrium. Smaller vessels can vary between individuals, but the major vessels are anatomically consistent.

The ovaries and uterus are drained by both the internal iliac and ovarian veins (OV). The IIV runs slightly medial and posterior to the internal iliac artery, joining the EIV to form the common iliac vein (CIV). Its tributaries are divided into parietal and visceral groups. Parietal tributaries include the superior and inferior gluteal, sciatic, sacral, ascending lumbar, and obturator veins. Visceral tributaries include the internal pudendal, middle hemorrhoidal, and vesicoprostatic plexuses in men, and the uterine, gonadal, and vesicovaginal plexuses in women. Valves are rarely present in the internal iliac veins (10% of cases in the main trunk and 9% in its tributaries).

The ovarian veins drain the pampiniform plexus, mesosalpinx, parametrium, and cervix, forming a rich anastomotic venous network with the paraovarian, uterine, vesical, rectal, and vulvar plexuses. Two or three branches form a single ovarian vein at the level of L4, with the left ovarian vein draining into the left renal vein (LRV), and the right ovarian vein in most

women draining directly at an acute angle into the anterolateral wall of the IVC, below the right renal vein (RRV). In up to 10% of women, the right ovarian vein may drain into the RRV instead of the IVC. Studies have shown that normal ovarian veins have an average diameter of less than 5 mm. Valves are present, mainly in the distal third of the vein. Valves are absent in 15% of left OVs and 6% of right OVs. When present, valves are incompetent in 40% of cases on the left and 35% on the right [1].

The left-sided predominance of PVCS can be explained by these anatomical features, as well as by the fact that the left ovarian vein is longer than the right, which impedes drainage in the upright position. Additionally, the left ovarian vein may be compressed by the sigmoid colon during constipation. Nonetheless, it should be noted that pelvic venous drainage is complex and venous anatomy can vary among patients. [5].

Etiology and Pathophysiology

The etiology of Pelvic Venous Congestive Syndrome (PVCS) remains poorly understood, and it is considered that multiple factors contribute to its pathogenesis. Pelvic venous insufficiency (PVI) can result from a combination of factors, including genetic predisposition, anatomical abnormalities, hormonal influences, valve dysfunction, obstruction of venous outflow by adjacent structures, and damage to the vein walls.

Many studies have indicated a connection between varicose veins and genetics, with some reports suggesting that up to 50% of varicose veins may have a genetic component. Congenital abnormalities of the vein wall may also exist, causing dilation and subsequent valve dysfunction.

Hormonal factors play a significant role in the development of PVCS. Estrogen increases nitric oxide production, resulting in venous dilation and weakening, which increases stress on the valves. Progesterone also contributes to weakening venous valves in the pelvic veins. Pregnancy is considered one of the major risk factors for PVCS due to increased circulatory volume in the pelvic veins, elevated flow through the ovarian veins (up to 60-fold), and increased intra-abdominal pressure caused by the gravid uterus, which further exacerbates ovarian vein reflux. Estradiol-induced venous dilation during pregnancy increases valve stress, ultimately leading to chronic venous insufficiency. The therapeutic use of vasoconstrictors has shown some efficacy in alleviating PVCS symptoms by increasing venous flow through compression, supporting the hormonal theory. Additionally, symptoms typically resolve completely after menopause.

Although valves are generally present in the distal segments of the main ovarian vein trunks (about 85% of cases), they are incompetent in 40% of cases on the left and 35% on the right ovarian vein [6]. The mechanisms by which venous valves become incompetent are not precisely defined. On one hand, there may be a primary change in valve structure leading to leakage, progressive reflux, and subsequent vein dilation. On the other hand, a primary structural abnormality in the vein wall may cause venous dilation, which distorts the valves and renders them nonfunctional [5].

PVCS can also result from obstruction of blood outflow from the ovarian veins. The most common cause of obstruction is compression of the left renal vein between the superior mesenteric artery and the abdominal aorta, known as Nutcracker syndrome. May-Thurner syndrome is another cause of obstruction, where the left common iliac vein is compressed by the right common iliac artery. This compression can sometimes lead to deep vein thrombosis. Abnormal uterine positioning with ovarian torsion can rarely cause obstruction. Additionally, endometriotic lesions, fibroids, postsurgical or infectious adhesions, hypervascular pelvic tumors, gestational trophoblastic neoplasms, ovarian tumors, and mesenteric tumors can also compress veins. Regardless of etiology, the final result of obstruction is the development of numerous refluxing varicosities, cross-

venous collaterals, and painful venous congestion. [1].

Prolonged venous dilation in varicose veins in PVI induces inflammation, which further damages the vessel walls, causing additional weakening and dilation of the veins and increasing reflux. Venous hypertension enhances the expression of matrix metalloproteinases, promoting the degradation of collagen, elastin, and endothelium, thereby impairing vascular tone regulation [7]. This process leads to further endothelial damage and inflammation.

Although venous distension generally should not cause pain, congestion and stretching of the ovarian and pelvic veins can activate pain receptors within the venous walls. Venous dilation leads to activation of nociceptors connected to C-afferent fibers, which have slow conduction velocities and mediate the sensation of dull, burning pain [2].

Venous dilation and inflammation also trigger the release of substance P and calcitonin gene-related peptide (CGRP), which further dilate the vessels and increase vascular wall permeability. Simultaneously, cytokines are released, enhancing inflammation and nociceptor activity [8].

Supporting evidence that dilation of pelvic veins activates pain receptors comes from clinical observations that gabapentin and amitriptyline—standard treatments for neuropathic pain—are more effective than opioids or nonsteroidal analgesics in alleviating pelvic pain. [9].

Diagnosis

The diagnosis of Pelvic Venous Congestion Syndrome (PVCS) remains challenging due to the lack of universally accepted criteria in imaging modalities and its heterogeneous presentation. Patients with PVCS typically first consult a general practitioner and/or gynecologist in primary care before being referred for further investigations and specialist consultation. Once more common causes of chronic pelvic pain—including endometriosis, pelvic inflammatory disease, interstitial cystitis, and fibroids—are excluded, the first diagnostic step is usually pelvic ultrasound (US) to visualize the blood vessels [1].

Various nomenclatures have been used to describe the diverse clinical presentation of pelvic venous insufficiency. A step toward better understanding of this condition is the recently established “Symptoms-Varices-Pathophysiology” (SVP) classification for assessing pelvic venous disorders, proposed by the International Working Group convened by the American Vein and Lymphatic Society. Although this classification may seem complex for routine clinical practice, it could help in more precise diagnosis, better selection of patients for therapeutic intervention, and generation of homogeneous samples for future research [10].

Imaging methods for diagnosis include non-invasive techniques such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), as well as invasive venography (VG).

Pelvic ultrasound is generally the first-line method for patients suspected of having PVCS. Ultrasound assesses pelvic anatomy and, using Doppler modes, allows visualization of blood vessels and evaluation of blood flow. Ultrasound can be performed transvaginally, transabdominally, or transperineally. Transvaginal ultrasound (TVUS) better excludes other gynecological conditions, provides improved visualization of pelvic venous plexuses, and allows dynamic assessment of blood flow through tortuous pelvic veins. Transabdominal and transperineal ultrasound, on the other hand, allow better visualization of longer vessels, such as the ovarian veins. Ultrasound can also be performed with the patient standing or performing the Valsalva maneuver, which accentuates venous filling and improves visualization of pelvic varices [2,5].

Ultrasound parameters that can be evaluated include: internal diameter of the largest pelvic vein (right and left), maximum diameter of the largest venous plexus (right and left), dilation and low velocity or reversed flow in the ovarian veins during Valsalva, enlargement of arcuate veins (vv. arcuate) in the myometrium, presence of crossed veins, maximum diameter of crossed veins in the myometrium, uterine volume, volume of the right and left ovary, and presence of polycystic ovaries (PCO).

The threshold for ovarian vein dilatation remains controversial, with different authors defining it between 5 and 8 mm. According to Park et al., the positive predictive value for a threshold diameter of the left ovarian vein was 71.2% at 5 mm, 83.3% at 6 mm, 81.8% at 7 mm, and 75.8% at 8 mm. [11].

Rocio Garcia-Jimenez et al. designed an ultrasound predictive model for identifying PVCS based on the presence of a pelvic vein or venous plexus measuring 8 mm or more, identified via transvaginal ultrasound (TVUS). This model was able to predict 79% of patients with PVCS, with good sensitivity (86.05%) and specificity (66.67%). Given its simplicity, relying on a single parameter, this model appears to be a feasible alternative compared to previously proposed predictive models [12]. Labropoulos et al. (2017) reported on the standardization and technique of ultrasound application in PVCS diagnosis using a transabdominal approach [13].

Computed tomography (CT) allows imaging in cross-sections and precise anatomical visualization. Magnetic resonance imaging (MRI) of the pelvis provides excellent image quality and high resolution, and unlike CT, does not involve radiation, making it safer for women of reproductive age. Diagnostic criteria for CT and MRI proposed by Coakley et al. include the presence of at least four ipsilateral tortuous parauterine veins of varying calibers, at least one vein with a diameter >4 mm, or an ovarian vein diameter >8 mm [14]. Both contrast-enhanced and non-contrast CT and MRI provide good sensitivity in diagnosing venous insufficiency. Osman et al. reported a sensitivity of 94.8% for CT and 96% for MRI [15].

Flow information in the veins can be obtained using MRI techniques such as phase-contrast velocity mapping (Phase Contrast MRI) or Time-Resolved MRA, which provide accurate information on whether flow in the ovarian vein is antegrade or retrograde. Yang et al. compared Time-Resolved MRA with conventional venography, showing that Time-Resolved MRA is an excellent non-invasive diagnostic tool for pelvic venous insufficiency, with no significant difference compared to conventional venography in determining the level of ovarian venous reflux [16].

Laparoscopy is not effective in detecting pelvic varices and is negative in 80–90% of PVCS patients because it requires Trendelenburg positioning and CO_2 insufflation, which increases intra-abdominal pressure and compresses (often masking) pelvic varices. However, laparoscopy allows visualization of other causes of chronic pelvic pain [2].

Transcatheter venography remains the gold standard for PVCS diagnosis. As an invasive procedure, it should be reserved for patients whose non-invasive imaging findings are inconclusive or for those planned for interventional embolization therapy [15]. Catheter-directed venography is performed by inserting a catheter via the jugular, brachial, or femoral vein to the renal, ovarian, common iliac, and internal iliac veins, followed by contrast injection. This technique allows measurement of pressure gradients, providing valuable information about the severity of pelvic venous pathology, as well as morphological assessment of the veins. The procedure is usually performed on an outpatient basis without hospitalization. A key advantage is that treatment can be performed in the same session. The main protocol begins with catheterization of the left renal vein, simultaneously measuring the pressure gradient to assess Nutcracker syndrome. The catheter is then moved to the left iliac vein to

evaluate May-Thurner syndrome. Subsequently, the ovarian veins are assessed, followed by the internal iliac veins [17].

Venographic diagnostic criteria for incompetent pelvic veins include an ovarian vein diameter >10 mm; congestion of ovarian, pelvic, vulvovaginal veins; and retrograde filling. [5].

Treatment of Pelvic Venous Congestion Syndrome (PVCS)

Conservative (Medical) Treatment

Medical management of PVCS is limited, as long-term efficacy data are lacking. Hormonal therapies that inhibit ovarian function, such as medroxyprogesterone acetate (MPA) and gonadotropin-releasing hormone (GnRH) agonists, have shown some efficacy, but their use is associated with multiple side effects. Dihydroergotamine has demonstrated temporary pain relief, but its effects are transient and accompanied by adverse events. Nonsteroidal anti-inflammatory drugs (NSAIDs) may alleviate symptoms but do not address the underlying condition [2].

Micronized purified flavonoid fraction (MPFF), a venoactive drug, has been investigated by Simsek et al., Tsukanov et al., and Gavrilov et al. All studies showed that 1000 mg of MPFF daily reduces the severity of pelvic symptoms such as pain, heaviness, and vulvar swelling due to pelvic varices [18,19,20]. Gavrilov et al. also demonstrated that doubling the dose (1000 mg twice daily) in the first month accelerates symptom resolution [21].

Compression garments are another conservative treatment option. In a study by Gavrilov et al., wearing compression shorts for 2 weeks reduced chronic pelvic pain, dyspareunia, and discomfort in 81.3% of patients. They also reduced leg heaviness and swelling. However, there was no effect on clinical symptoms of vulvar varices. Elastic stockings did not show clinical improvement or enhanced venous drainage [22].

Non-conservative treatment includes surgical intervention and minimally invasive endovascular therapy. Earlier surgical approaches, such as left ovarian vein resection or hysterectomy with unilateral or bilateral adnexectomy, were associated with high recurrence rates, residual pain, longer hospital stays, and higher morbidity compared to endovascular approaches.

In a randomized controlled trial by Chung et al., ovarian vein embolization was significantly more effective than hysterectomy with unilateral or bilateral salpingo-oophorectomy 12 months post-treatment [23].

The first report of embolization as a treatment for PVCS was published by Edwards in 1993. According to the Society for Vascular Surgery and the American Venous Forum, embolization is recommended with a 2B level of evidence for PVCS treatment [17]. Embolization is usually performed after unsuccessful medical therapy but is increasingly used as a primary treatment. Indications generally include women with chronic pelvic pain and/or dyspareunia, severe labial or perineal varices, or lower limb varices, with confirmed pelvic venous insufficiency, typically verified by venography.

There is no standardized protocol for endovascular PVCS treatment. Techniques, vascular access sites, and embolic materials (sclerosants, coils, plugs) vary across publications [2]. Clinical improvement after embolization ranges from 47% to 100% in different studies [2]. The debate continues over whether unilateral or bilateral embolization should be performed; some clinicians perform only unilateral ovarian vein embolization, while others perform complete bilateral embolization [2].

If a hemodynamically significant stenosis is present, it should be corrected. This may include stenting the left common iliac vein in May-Thurner syndrome or the left renal vein in Nutcracker syndrome, as well as any other catheter-accessible site of pelvic venous obstruction. Stenting of the left renal

vein carries a high risk of migration to the vena cava and heart due to the vein's short length and diameter changes during posture changes or Valsalva maneuvers [24]. The main risk of endovascular stenting failure is stent occlusion. Duration of post-procedural antithrombotic therapy varies between studies [2].

Complications are generally rare and minor, including allergic reactions, puncture site hematoma, local thrombophlebitis, vessel perforation, embolic migration, and recurrence of symptoms. PVCS symptoms may recur after ovarian vein embolization due to reflux from other venous tributaries. Post-embolization syndrome occurs in approximately 20% of patients and is characterized by increased pelvic pain, low-grade fever, and tenderness around the embolized vein, usually managed with NSAIDs. A potentially serious complication is migration of the coil or vascular plug to the pulmonary artery, which is typically successfully retrieved endovascularly. [2].

CONCLUSION

Pelvic venous congestion syndrome (PVCS) is a common cause of chronic pelvic pain in women, but due to insufficient awareness, this syndrome is often unrecognized and remains undiagnosed. The symptoms can be nonspecific and are frequently underestimated. Diagnosing PVCS is very challenging and complex, yet equally important for implementing appropriate and targeted treatment. Globally accepted diagnostic algorithms that allow for an objective diagnosis are still lacking. Considering that most patients with chronic pelvic pain initially consult general practitioners or gynecologists, it is important to always consider this syndrome in the absence of other causes. Additional education of gynecologists in the use of ultrasound for diagnosing pelvic venous insufficiency (PVI) and familiarity with diagnostic criteria would also be beneficial, as ultrasound is the first-line method in PVCS diagnosis. There is also a need for validated imaging diagnostic criteria.

Regarding treatment, endovascular embolization appears to be an effective method; however, future randomized studies are needed to establish clear protocols for managing embolization..

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Vol. 50 (2025) No. 3&4 TIMOK MEDICAL GAZETTE Review article

OVARIAN TORSION - review paper

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Summary: The largest number of ovarian torsions is seen in the reproductive period, around 71%, but it also occurs in fetuses and neonates, premenarchal girls, pregnant women, and postmenopausal women. Although the true incidence of torsion is still unknown, data show that torsion accounts for 2.7% of surgical interventions, making it the fifth most common condition requiring emergency surgery. To understand how torsion occurs, we must understand the anatomy of the supporting structures of the uterus and ovaries. The ovary is a mobile structure in itself, suspended from the pelvic wall by the infundibulopelvic ligament and attached to the uterus by the utero-ovarian ligament. Torsion occurs as a result of partial or complete rotation of the adnexal supporting structures, which leads to partial or complete obstruction of ovarian blood flow, resulting in ovarian ischemia, which leads to necrosis, local hemorrhage, and loss of function. Torsion can occur in women of all reproductive ages, but the highest percentage of ovarian torsions occurs in women in the reproductive period with adnexal changes such as functional ovarian cysts and benign tumors. The most common symptom is pain, which may be accompanied by nausea and vomiting. Physical examination may reveal low-grade fever, abdominal tenderness, abdominal pain, and a pelvic adnexal mass. Diagnosing ovarian torsion usually requires a combination of medical history, clinical examination, and imaging methods. Early diagnosis and surgical treatment are essential to preserve ovarian and tubal function and to prevent more serious morbidity..
Keywords: Ovarian torsion, ovarian cyst, benign tumor, acute abdomen

INTRODUCTION

The most common conditions that lead to acute pain in gynecological practice include ectopic pregnancies, pelvic inflammatory disease, ruptured ovarian cysts, ovarian torsion, torsion and degeneration of uterine leiomyomas, and spontaneous miscarriages. The largest number of ovarian torsions is seen in the reproductive period, around 71% [2], but it also occurs in fetuses and neonates, premenarchal girls, pregnant women, and postmenopausal women. Although the true incidence of torsion is still unknown, data show that torsion accounts for 2.7% of surgical interventions, making it the fifth most common condition requiring emergency surgery [2]. Another study found that 15% of surgically treated adnexal masses are in torsion [3]. Timely diagnosis is important both for preserving ovarian function and for preventing subsequent comorbidities..

PATHOGENESIS AND RISK FACTORS

To understand how torsion occurs, we must first understand the anatomy of the supporting structures of the uterus and ovaries. The ovary is a paired intraperitoneal organ with two primary functions: the production of sex hormones, and the development and release of the oocyte during ovulation, as well as the formation of the corpus luteum, which provides sufficient hormonal support to early pregnancy until placental function is established. What is specific and remarkable about the ovary is that it can increase its volume several hundred times during a woman's reproductive period without pathological clinical manifestations [4].

The ovary is a mobile structure, suspended from the pelvic wall by the infundibulopelvic ligament (also called the suspensory ligament of the ovary), through which the ovarian artery passes, and attached to the uterus by the utero-ovarian ligament (ligamentum ovarium proprium), through which the ovarian branch of the uterine artery passes. In addition to providing support, these ligaments also serve a nutritive role, as the blood vessels supplying the ovary run through them, ensuring dual vascularization of the ovary [5].

Torsion occurs as a result of partial or complete rotation of the adnexal supporting structures, during which the ovary and fallopian tube rotate around both the infundibulopelvic and utero-ovarian ligaments, resulting in partial or complete obstruction of ovarian blood flow [6,7,8]. The thin walls of the veins are more prone to complete occlusion compared to the muscular walls of arterial vessels. Continuous arterial inflow without venous outflow leads to edema with visible ovarian enlargement. Further vascular compression results in ovarian ischemia, leading to necrosis, local hemorrhage, and loss of function [9]. Most often, both the ovary and the fallopian tube undergo torsion simultaneously, although isolated torsion of the ovary or tube may occur, referred to as partial torsion. Torsion involving paraovarian or paratubal cysts has also been described [6]. The right ovary is more frequently affected than the left, possibly because the right utero-ovarian ligament is longer, and the presence of the sigmoid colon prevents torsion on the left side [8,10]. Bilateral asynchronous ovarian torsion is also possible, though rare [11]. The severity of symptoms and morphological ovarian changes depends on the type and degree of vascular occlusion. Based on anatomical features and clinical findings, we can define risk factors for adnexal torsion. Greater ovarian mobility is associated with torsion in premenarchal girls, who have elongated infundibulopelvic ligaments. In this population, more than half of patients have morphologically normal ovaries. After this premenarchal period, with puberty, the incidence of ovarian torsion decreases due to shortening of the infundibulopelvic ligaments. Risk factors in premenarchal girls may also include the presence of functional cysts or benign tumors, most commonly teratomas and cystadenomas [12,13,14].

Ovarian torsion has also been described in the fetal period (ultrasound may monitor cyst growth and secondary changes such as hemorrhage, calcifications, or resorption) and in neonates [15].

The highest percentage of ovarian torsions occurs in women of reproductive age with adnexal changes such as functional ovarian cysts and benign tumors [8,9,16]. Malignant tumors and endometriotic cysts are rarely the cause of ovarian torsion. In case series, the percentage of malignant ovarian tumors associated with torsion is reported to be below 3%. This is because such lesions cause peritoneal reactions and adhesions that fix the mass, thereby limiting its mobility [17]. More than 80% of patients with ovarian torsion have ovarian masses larger than 5 cm in diameter. The size of the ovarian mass correlates with the risk of torsion. In a series of 87 case studies, ovarian masses ranged widely from 3 to 30 cm, with an average of about 9.5 cm [18]. About 10–22% of ovarian torsions occur during pregnancy. The incidence is somewhat higher between the 10th and 17th weeks of gestation in the presence of ovarian masses larger than 4 cm. Ovulation, the corpus luteum, and ovulation induction in infertility treatment may cause ovarian hyperstimulation syndrome, with multiple large cystic ovarian changes that are prone to torsion. Polycystic ovary syndrome is also a risk factor [19]. On the other hand, in patients who have undergone a surgical procedure, the incidence of ovarian torsion is about 2–15%, typically due to strangulation of the ovarian pedicle around an existing adhesion. Recurrent torsion has also been described, and studies show that individuals who have experienced ovarian torsion once are at increased risk of developing torsion again—either of the same ovary ("salvage ovary") or of the contralateral ovary. [8].

Clinical presentation and clinical findings

Ovarian torsion caused by the presence of an adnexal mass results in a variety of symptoms, clinical signs, and presentations. The most common symptom is acute, sharp pain in the lower abdomen or pelvis, accompanied by nausea and vomiting (70%) in women of reproductive age, in the presence of an adnexal mass or enlarged ovaries in PCOS or ovarian hyperstimulation, or in women with a history of prior ovarian torsion [17,18,20]. Some patients experience only nausea without vomiting. Abdominal pain is most often intermittent, colicky in nature, with gradual intensification and relief, although it may also be continuous. The pain arises secondarily due to occlusion of the vascular pedicle and is refractory to analgesics. It may radiate to the inguinal region or flank. Premenarchal patients may report diffuse abdominal pain, as they often find it difficult to localize the discomfort. In this group, vomiting is the most common symptom in the absence of adnexal pathology—this represents a vagal reflex response due to peritoneal irritation. In neonates, torsion may present with

feeding difficulties, abdominal distension, vomiting, and irritability. Ovarian torsion without infectious pathology may also be accompanied by low-grade fever. The low-grade fever is explained by necrotic changes in the torted ovary and occurs in 2–20% of patients. Physical examination may reveal low-grade fever, abdominal tenderness, abdominal pain, and a pelvic adnexal mass. A further diagnostic challenge is that 30% of patients—especially those in the premenarchal period—may have neither abdominal pain nor abdominal tenderness. [17,18,21-24].

Diagnosis

The diagnosis of ovarian torsion most often requires a combination of anamnestic data, clinical examination, and imaging methods. The first approach to the patient is the physical examination and taking the medical history. Anamnestic data may indicate a recent diagnosis of an adnexal mass, recurrent abdominal pain, and low-grade fever. In children aged 2–14 years, with high sensitivity and positive predictive value, the Bolli score can be applied. The Bolli score includes only the patient's clinical data but not imaging methods and identifies three useful clinical variables on the basis of which the ovarian torsion score is established: the age of the child, the duration of pain, and vomiting. –Number of points – number of years, minus three points if vomiting is present, plus one point if the duration of pain is longer than 12 hours. The cut-off value of the Bolli score in girls is 11.5, a lower score indicating a higher probability that ovarian torsion is present. [25,26].

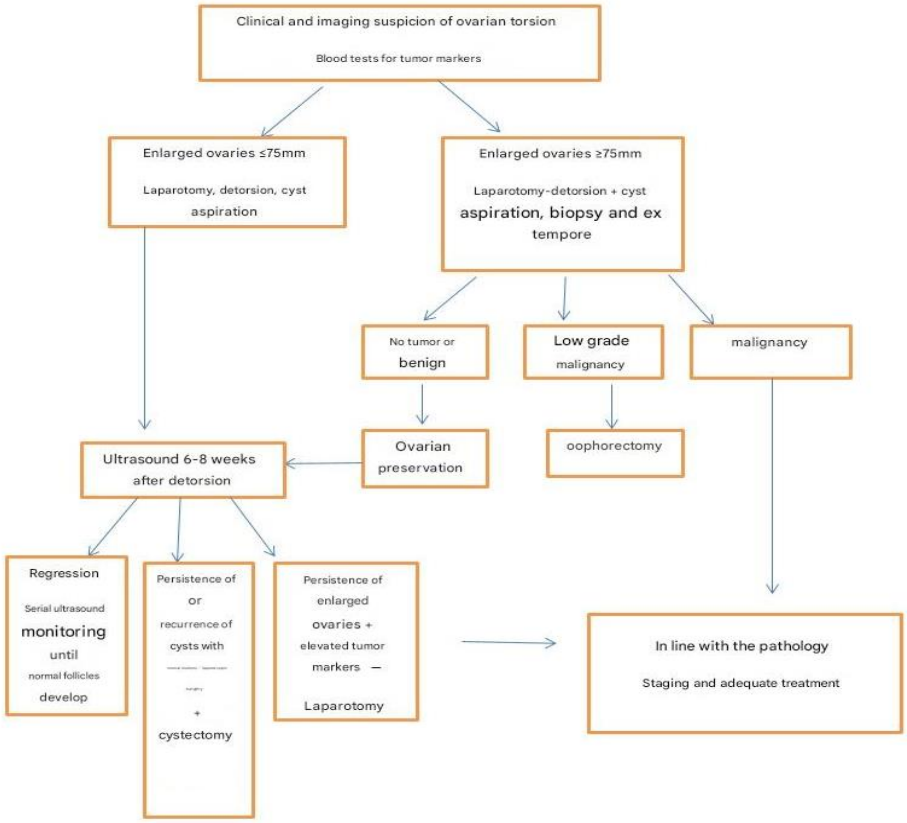
LABORATORY TESTING should include hematocrit, leukocyte count, human chorionic gonadotropin (HCG), electrolytes, and inflammation parameters—C-reactive protein (CRP) [2,22]. Laboratory analyses may be completely normal, may indicate anemia in the case of corpus luteum rupture, or leukocytosis and elevated CRP due to tissue necrosis and consequent inflammation. The level of interleukin 6 is also elevated and indicates increased oxidative stress in torsion, but it is also a nonspecific sign of inflammation and is not routinely performed in our clinical practice [27,28]. Determining tumor markers has not proven to be sufficiently sensitive or specific, although the elevation of certain tumor markers may indicate the nature of the torted adnexal mass. The physical exam is focused on abdominal palpation in order to detect a tumor mass and assess the presence of peritoneal irritation. Imaging studies are the most important. Ultrasound is the first-line diagnostic tool [29,30].

In the pediatric population, transabdominal ultrasound with a full bladder is the initial imaging method for evaluating torsion. The sensitivity of transabdominal ultrasound in the pediatric population is 92–93%, with a specificity of 96–100%. In adult women, transvaginal ultrasound shows excellent specificity but variable sensitivity, ranging from 35–85% [31]. What is monitored on ultrasound is ovarian volume, presence of edema, presence of an adnexal mass, presence of free fluid, and color Doppler of ovarian or tumor mass blood vessels. The presence of a difference in ovarian volume with its displacement is a pathognomonic sign of ovarian torsion. Another sign is the presence of edema of normal ovarian tissue. In the literature, it is described as the presence of peripheral follicles with hyperechogenic halos in the ovary without cystic changes or tumors — strings of pearls. The presence of ovarian edema should not be mistaken for the presence of a solid ovarian tumor.

The torted ovary may be rounder and enlarged compared to the contralateral one due to swelling of vascular and lymphatic vessels. There may be normal, reduced, or completely absent blood flow through the vessels of the torted ovary [31–34]. The whirlpool sign is a highly sensitive and specific sign for the diagnosis of ovarian torsion. The whirlpool sign indicates the twisted vascular pedicle, and Doppler sonography reveals circular blood vessels within the mass [32]. Finally, a small amount of free fluid may be present in the pouch of Douglas [31]. The greatest diagnostic challenge is torsion without twisting of the ipsilateral ovary. It has been shown that 31% of all torsions are incomplete adnexal torsions. A useful sign of torsion involving only the tube but not the ovary is the presence of three or more cysts in one row [35]. The combination of free fluid in the pelvis, an enlarged ovary, and vascular abnormalities increases the sensitivity and specificity of ultrasound findings. CT of the abdomen and pelvis shows high sensitivity and specificity in the evaluation of suspected torsion [36] and may show an enlarged ovary, its displacement and pulling of the uterus to that

side, thickening of the cystic mass, ascites, thickened walls of the tube [36,37]. The definitive diagnosis is made in the operating room by direct visualization of the specimen..

Algorithm 1. Algorithm for management in cases of clinical and imaging suspicion of ovarian torsion.



Treatment and assessment of ovarian viability

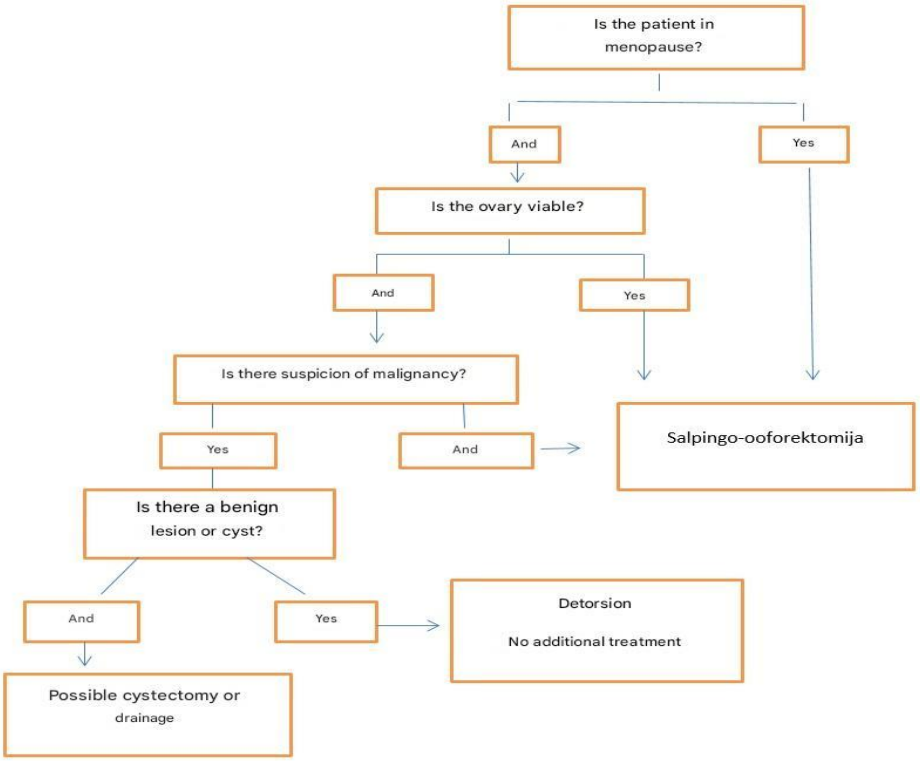
Treatment involves surgical management and at the same time confirmation of the diagnosis. Early diagnosis and surgical therapy are necessary in order to protect ovarian and tubal function and to prevent more serious morbidity. Minimizing the total time during which the ovary is in ischemia is a key component of therapy, but the time required for ovarian necrosis to occur is unclear. [37,38].

Picture 1. Surgery of a torted ovarian fibroma (Dr. Janković, General Hospital Pirot))

As long as the venous and lymphatic vessels are occluded, the patient may have symptoms for some time before the arterial vessels become occluded [30,40,41]. In a retrospective study of the pediatric population, the median time to save the ovary before

detorsion was 10.8 hours. If detorsion is performed within the first 8 hours, the ovary is preserved in 40% of cases, and within the first 24 hours in 33% [42]. This finding is consistent with data showing that in women, the percentage of preserved ovaries is 30% if surgery is performed within the first 24 hours from the onset of symptoms [43,44]. Different studies show varying times from symptom onset to detorsion in order to preserve the ovary. Animal studies have shown that necrosis can occur 36 hours or more after occlusion. Pediatric and adult populations show good long-term outcomes after detorsion of either hemorrhagic or ischemic ovaries, with normal follicle production later in life in 90–94% of cases described [45,46]. There are two surgical treatment methods — laparoscopy and laparotomy. Laparoscopy represents a reasonable alternative. The benefits of laparoscopy include reduced need for analgesics, early mobilization, cosmetic advantage, and earlier discharge to home care. An additional advantage is that laparoscopic ovarian cystectomy is associated with a lower incidence of postoperative adhesions compared to laparotomy [47]. Laparotomy is recommended when a malignant process is suspected. What is essential is the assessment of ovarian viability and preservation of its function. The only way to assess the viability of the ovary is by gross visual inspection. Conventionally, a dark and enlarged ovary may be only in venous or lymphatic congestion and may appear nonviable, but there is a substantial probability that it is a viable ovary that can regain function after detorsion [46]. There are other methods to assess ovarian viability, such as injecting fluorescein and observing the flow under ultraviolet light [48]. Another method is ovarian bivalving, i.e., laparoscopically making an incision in the ovary with an electric hook (L-hook) after detorsion and observing whether there is blood flow at the cut surface. This also serves a therapeutic purpose by reducing pressure caused by venous and lymphatic congestion [49]. There is no precisely determined time for ovarian necrosis to occur. A definitive sign of ovarian necrosis is a gelatinous formation that disintegrates upon manipulation. What is expected from surgical treatment? Ideally, detorsion [50], detorsion with oophoropexy, ovarian cystectomy (recommended for benign cysts after detorsion), or salpingo-oophorectomy in cases of suspected malignancy, necrotic ovaries, and postmenopausal women.

Algorithm 2. Procedure in menopausal women



CONCLUSIONS:

- Ovarian torsion mainly affects women of reproductive age, but a significant percentage also occurs in the premenarcheal period, in pregnant women, and in postmenopausal women.
- • Ovarian torsion occurs due to complete or partial rotation of the ovary and fallopian tube, leading to obstruction of vascular flow.
- • Crucial factors for ovarian torsion include the presence of an ovarian mass in women of reproductive age.
- • Almost 90% of women experience abdominal pain that begins suddenly, is sharp, and intermittent in nature.
- • Up to 70% experience nausea and vomiting.
- • Nearly one-third of patients with torsion have no abdominal or pelvic pain on examination.
- • Although ultrasound is used as the primary diagnostic modality in the evaluation of ovarian torsion with high specificity, a normal ultrasound finding cannot effectively rule out the diagnosis.
- • CT of the abdomen and pelvis with IV contrast can be helpful in diagnosis.
- • In the evaluation, findings for reduced or absent ovarian enlargement, peripheral displacement of follicles, enlarged ovaries with follicular stroma, and thickened fallopian tube are considered significant..

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MALIGNANT PLEURAL MESOTHELIOMA – CASE REPORT

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Summary: Introduction. Malignant pleural mesothelioma (MPM) is a rare and highly aggressive tumor. Its occurrence is causally linked to asbestos exposure, which is the leading etiological factor contributing to the development of the disease in more than 80% of cases. It occurs after inhalation of microscopic asbestos mineral fibers, following a long latent period. The time from asbestos exposure to tumor onset is usually several decades. The disease is more common in men. The therapeutic approach is based on a multimodal strategy, combining surgery, chemotherapy, and radiotherapy. Regardless of the treatment applied, the prognosis is always very poor. The aim of this paper is to present the basic characteristics of MPM and to increase fundamental knowledge about the harmful effects of asbestos in the development of the disease. **Case presentation:** A case of malignant pleural mesothelioma in a 64-year-old male patient is described. The main symptoms included dyspnea, cough, and fatigue on minimal exertion. Physical examination of the lungs revealed absent breath sounds on the left side. The initial chest X-ray indicated the presence of a massive left-sided pleural effusion. After drainage of the left pleural space, 3000 ml of fluid was evacuated. A video-assisted thoracoscopic surgery (VATS) was subsequently performed, along with partial pleural decortication and biopsy, which confirmed the diagnosis of MPM, epithelioid subtype. The most likely asbestos exposure occurred 30 to 35 years earlier. **Conclusion**: The presented case of MPM describes a patient who initially exhibited typical nonspecific symptoms, a characteristic unilateral pleural effusion, and later severe chest pain with rapid disease progression. The disease is most often diagnosed at an advanced stage. A history suggesting possible asbestos exposure during the patient's lifetime may raise suspicion and contribute to an earlier diagnosis, at a stage when therapeutic options are somewhat greater..

Ključne reči: male sex, malignant mesothelioma, mineral fibers, pleura, asbestos, prognosis.

INTRODUCTION

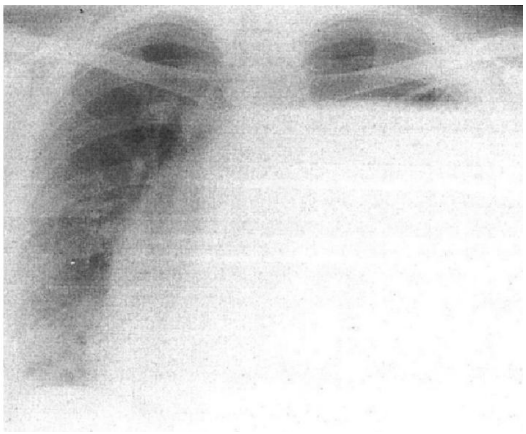
Malignant mesothelioma is a relatively rare but very aggressive tumor. It represents a multifactorial disease in whose development the following factors play a role: asbestos, Simian virus 40, and radiotherapy [1]. It has not been proven that smoking causes the occurrence of MPM, but it contributes to its development. According to data from the literature, its occurrence is causally related to asbestos exposure as the leading etiological factor that contributes to the development of the disease in more than 80% of cases. It appears after inhalation of microscopic asbestos mineral fibers suspended in the air, after a long latent period of several decades. It has a much higher incidence in men, which is explained by the fact that men are more often engaged in occupations that are "risky" in terms of asbestos exposure. Occupational exposure to asbestos has been the subject of numerous studies. Such an association of MPM with occupation is most likely the consequence of not implementing occupational safety measures. A particularly concerning fact is the occurrence of mesothelioma in family members of these workers. The disease also more frequently appears in places where mines of this material are located, because exploitation leads to contamination of the environment (air) and exposure of the population to asbestos (endemic areas) [2]. As a carcinogenic material, asbestos was banned in all European Union member states in 2005, while Serbia introduced a ban on the use of asbestos in all products in 2011, and a Regulation on handling waste containing asbestos was also prescribed. [3].

CASE REPORT

The patient is a 64-year-old male. A retired machine locksmith. Smoker for over 40 years, about 20 cigarettes per day, rarely consumes alcohol. In his personal medical history previously healthy, without other comorbidities. The patient states that he felt the

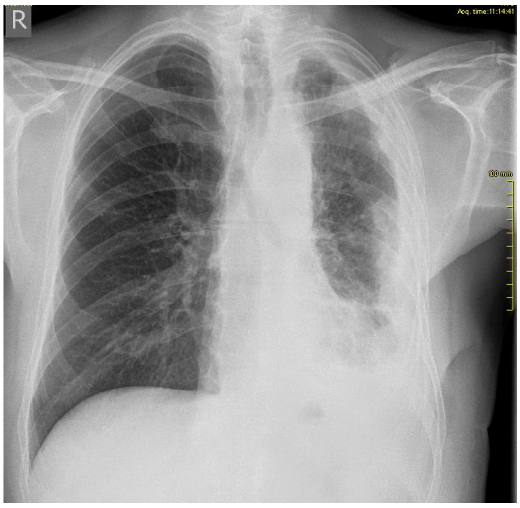
first symptoms at the beginning of June 2018. The main complaints are shortness of breath, a feeling of choking, and fatigue on minimal exertion. Cough has been present for several months before that, since March 2018. Elevated blood pressure for the past two weeks, BP 160/100 mmHg, previously normal blood pressure. Evaluated by an internist, received antihypertensive therapy and was further referred to a pneumophysiologist. Auscultation of the lungs on the left reveals completely absent breath sounds, without accompanying sounds, while in the other parts of the lungs the breath sounds are normal. Blood oxygen saturation is 97%.

Figure 1. Initial chest radiograph



Chest radiography (Figure 1) indicates a left hydropneumothorax, with an infraclavicularly present hydro-air level. The cardiac silhouette is displaced to the right. All laboratory and biochemical analyses are within reference values. After the basic laboratory and diagnostic examinations performed at the Health Center Knjaževac, the patient was further referred to the Special Hospital for Pulmonary Diseases Ozren on 26.06.2018. Two days later he was transferred to the Clinic for Thoracic Surgery, University Clinical Center Niš, for further treatment, where he was hospitalized several times in the following period. During the first hospitalization at this clinic, an initial drainage of the left pleural space was performed, with approximately 3000 ml of fluid evacuated. Cytological and bacteriological analyses did not indicate the presence of tumor cells or infection.
During the next hospitalization, surgery was performed on 24.07.2018., video-assisted thoracoscopy (VATS), partial pleural decortication and biopsy were done, and the material was sent for histopathological examination. In the histopathological report dated 11.09.2018., malignant pleural mesothelioma, epithelioid variant, was diagnosed. On the follow-up PA chest radiograph from 16.09.2018. (Figure 2), the left hemidiaphragm and left costophrenic angle are obscured by a laterally ascending shadow—pleural effusion is present. The remaining part of the lung parenchyma on the left shows reduced transparency.

Figure 2. Control radiography of the chest



In the conclusion of the chest MSCT dated 24.09.2018: in the pleural cavity on the left, a heterogeneous-density lesion is present diffusely, with denser fluid content, accompanied by compressive atelectasis and a soft-tissue component; the described lesion primarily corresponds to a neoplastic process with empyema. In the remaining lung parenchyma, micronodular changes and mediastinal lymphadenopathy are present. In the bony structures, apart from degenerative changes, there are no other MSCT findings.

According to the decision of the Pulmonology Oncology Council from 25.09.2018, treatment with first-line chemotherapy using the pemetrexed–cisplatin regimen was planned. In the meantime, further disease progression occurred. Poor appetite and progressive weight loss were present. The appearance of central neurological symptoms was suspected, including impaired

communication, occasional disorientation to time, instability, and intermittent loss of sphincter control (occasional urinary incontinence). Pain was constantly present. From analgesic therapy, Ibuprofen 600 mg tablets 2×1 and Tramadol 50 mg tablets 2–3×1 were administered, but due to insufficient analgesic effect, the use of a Fentanyl transdermal patch 25 micrograms/h was soon initiated. The patient was hospitalized at the Oncology Clinic, University Clinical Center Niš, on 15.10.2018. Laboratory and biochemical analyses showed azotemia and hypercalcemia (urea 16.4 mmol/L, creatinine 179.4 μmol/L, Ca 4.26 mmol/L). Due to clinical deterioration and poorer performance status, chemotherapy was not indicated. Because of the elevated serum calcium levels, a decision was made to administer bisphosphonates, and urgent treatment was initiated. Therapy with Zoledronic acid 4 mg ampoule was administered without complications. However, progressive central neurological deterioration ensued, and the patient died before the first cycle of the planned systemic therapy.

Due to the specific nature and diversity of his occupations, asbestos exposure was likely present on multiple occasions throughout his life. However, the most probable critical exposure to asbestos may have occurred 30 to 35 years earlier..

DISCUSSION

Malignant mesothelioma can have different localizations and arises from mesothelial cells of serous membranes that line body cavities and organs (visceral or parietal pleura, peritoneum, pericardium, or, rarely, the coverings of other organs, e.g., the tunica vaginalis of the testis). Most commonly diagnosed is malignant pleural mesothelioma (MPM), accounting for over 70% of cases. The tumor appears after a very long latent period of several decades. The time from asbestos exposure to tumor development is at least 25 years, and according to some authors, more than 50 years. Due to this long latent period, MPM is most often diagnosed in patients over 60 years of age. Among patients with confirmed high-risk occupations, the most common were machine-fitters, as in our patient [1,4].

Asbestos includes six naturally occurring silicate minerals. It consists of soft, thin, silky fibrous crystals. There are two types of asbestos fibers: amphibole (most commonly used: crocidolite or blue; amosite or brown asbestos; fibers are long, thin, and straight – needle-like) and serpentine (chrysotile or white asbestos; fibers have a serpentine shape) [5]. Asbestos was widely used worldwide, especially in the second half of the last century. Due to its favorable physical properties, it had broad applications: it is a good conductor of heat, does not burn or carbonize, and is durable. All forms of asbestos fibers can be responsible for disease development. Some forms are more pathogenic than others. Thinner and longer fibers have the greatest carcinogenic potential. All types of asbestos are very stable and do not degrade spontaneously over time; however, processing or damage produces asbestos dust. This dust is easily inhaled and reaches the alveolar sacs. The exact mechanism by which asbestos fibers reach the pleura and mesothelial cells is not fully clarified. Over a long period, these fibers cause chronic inflammation, fibrosis, and malignant alterations. Oncogenesis is not fully understood. MPM most likely arises as a result of inactivation of tumor suppressor genes. The most frequent changes are loss of function of the CDKN2A tumor suppressor gene, NF2 inactivation, and mutation or deletion of the BAP-1 tumor suppressor gene (BRCA1-associated protein 1) [6]. All these factors together contribute to the development of MPM.

Symptoms of mesothelioma vary depending on the localization and stage of the disease. After a long latent period, initial symptoms are usually nonspecific and mild. In pleural mesothelioma, complaints include breathing difficulties, progressive dyspnea, and rapid fatigue with minimal exertion. In our patient, all these symptoms were present. The cough was dry and exhausting, and hemoptysis (coughing up blood) may occur. Our patient did not have hemoptysis, but the cough was present. Some symptoms may result from pleural effusion. Elevated blood pressure is not described as a symptom of MPM, but in our patient, it was likely a consequence of massive left-sided pleural effusion. Pleural effusions can be massive and often recurrent. During effusion drainage, transient relief occurs, followed by pain. Chest pain is the leading symptom of MPM and is thought to result from tumor infiltration into surrounding structures. Severe chest pain was also present in our patient. Neurological symptoms in the patient may be a possible consequence of disease dissemination to the CNS.

Some general symptoms, such as malaise, general weakness, fatigue, loss of appetite, and weight loss, were also present in our patient during the later course of the disease. Occasionally, fever, chills, and night sweats may occur, usually in advanced stages of the disease.

Standard chest radiography is a first-line diagnostic method, although it is not sufficiently sensitive or specific. A common finding on radiography is the presence of a unilateral pleural effusion. Cytological analysis of punctured pleural fluid has a sensitivity ranging from 13% to 75%, but it may be negative or false-negative. In the described patient, it was negative. Chest CT is an indispensable diagnostic procedure that provides valuable information about the pleura (thickening, calcifications), characteristics of effusions (if present), and the condition of mediastinal lymph nodes and organs.

Percutaneous biopsy is both a diagnostic and therapeutic procedure for MPM. Video-assisted thoracoscopy (VATS) is the most reliable method, providing an adequate sample for morphological and immunohistochemical analysis. Macroscopically, these tumors appear as diffuse pleural thickening. Pathohistological diagnosis is the gold standard. Histologically, tumors are divided into subtypes: epithelioid, sarcomatoid, and biphasic, which consists of a mixture of the two types [1,4]. In our patient, the diagnosis of epithelioid MPM was established, which is the most frequent type, accounting for about 60% of cases. It has a better prognosis, responds better to therapy, and has longer average survival. Although there are three histological subtypes of MPM, the WHO in 2021 proposed a complex and comprehensive classification of pleural and pericardial tumors [7,8], taking into account histological characteristics, prognosis, disease extent, BAP1 tumor suppressor gene immunohistochemistry, CDKN2A homozygous deletion, and other factors.

The therapeutic approach is based on a multimodal strategy, combining surgery, chemotherapy, radiotherapy, and immunotherapy. Despite significant progress in recent years, treatment options remain limited. Current therapeutic modalities prolong survival but do not provide complete cure.

Operability depends on tumor size and the patient's general condition. Stages I to IIIa are operable if the tumor is still localized and of the epithelioid type. In later stages with metastases, surgery has a palliative benefit.

Chemotherapy is the most commonly used treatment modality for mesothelioma and is applied in all stages. Pemetrexed and cisplatin constitute the first-line chemotherapy regimen. Some patients may respond better to other recommended combinations, such as pemetrexed with carboplatin, or cisplatin with gemcitabine [9]. Radiotherapy has most often been applied palliatively to relieve symptoms in later stages of disease, although technical advances have allowed significant improvements in MPM management [10].

In recent years, immunotherapy with monoclonal antibodies has been implemented. Nivolumab in combination with ipilimumab is indicated as first-line treatment for patients with unresectable MPM. Treatment continues until disease progression, unacceptable toxicity, or for up to 24 months. Patients treated in this way have shown significant improvement [11].

Malignant pleural mesothelioma is a highly aggressive tumor. The disease is incurable in later stages, and the prognosis is always very poor. Expected survival is less than 18 months from the onset of initial symptoms, while survival for advanced disease without therapy is 6–8 months.

According to a study conducted in 2019 in an endemic area of Turkey [2], postoperative survival results showed a median survival period of 19.6 months. Among 13 patients, the longest survival of 32 months was observed in a patient who underwent postoperative hyperthermic chemotherapy after pleural decortication..

CONCLUSION

There are anamnesis data that deserve attention: sex, age (over 60 years, due to the long latent period), occupation, and smoking history. The described case represents a typical patient with MPM, who initially presented with typical nonspecific symptoms such as cough, dyspnea, and fatigue, along with a characteristic unilateral pleural effusion, and later developed severe chest pain and rapid disease progression. The disease is most often diagnosed at an advanced stage. Anamnestic data regarding possible asbestos exposure during life may raise suspicion and contribute to an earlier diagnosis, at a stage when therapeutic options are

somewhat greater and may, for a limited time, prolong survival. The most important measure is primary prevention: to prevent asbestos exposure or to safely remove asbestos-containing material. MPM is a rare disease, but it should be considered in the differential diagnosis, as it is a highly aggressive tumor..

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TREATMENT OF PATIENTS WITH PERIODONTITIS (PERIODONTAL DISEASE) THROUGH HISTORY

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Summary: Preserved biological remains of teeth and jaws from prehistoric times, showing signs of periodontal tissue diseases, indicate that these diseases are as old as humanity itself. There are numerous evidence of human interest in oral healthand it dates back centuries ago. The oldest civilizations and their understanding of dental pain and therapeutic procedures form the foundation of the science we learn and know today. Starting from ancient Greece, Rome, and the Renaissance, where the brightest minds tackled human issues, earning the gratitude of generations that followed, to modern times where maintaining oral health is considered one of the vital components of a healthy human organism. This medical article aims to examine the development of periodontology as a branch of dentistry, compare the therapies and perceptions of those who studied it, and attempt to summarize the achievements of dental science as we know it today.

Keywords: History of periodontitis treatment, periodontal tissue, gingiva, the periodontal ligament, tooth cement, alveolar bone, alveolus

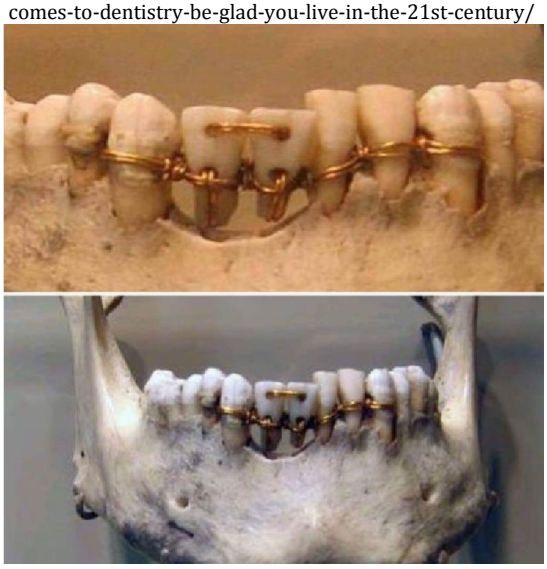
INTRODUCTUON

The, periodontal tissue (supporting structure of the tooth) consists of the gingiva, alveolar bone, the periodontal ligament, and root cementum. These tissues, despite their histological differences, have a common function: to firmly hold the connection of the tooth within the alveolar bone. Today, material, written and biological remainsfrom earlier periods are available, allowing us to create a clearer imageof how the science evolved regarding the treatment of periodontal diseases, from ancient times to the present.

The structure of periodontal tissues, due to its high resistance, has enabled their preservation as biological material for studying ancient civilizations. Paleostomatology or paleopathology is a specialdiscipline developed with the aim of studying the tissues of teeth and the periodontal tissues [1]. These findings have helped historians understand daily life, nutrition, and treatment methods for periodontal diseases, which largely explain the development level of ancient civilizations. Tooth decay, enamel hypoplasia, tooth and jaw fractures, and complications such as abscesses are among the diseases observed in excavated skull bone remains from prehistoric times [24]. In ancient Mesopotamia, medicine was regulated by Hammurabi's Code, the most famous and oldest legal document written in cuneiform script. From it, we can conclude that people who treated others' ailments existed thousands of years BC. Religion had a significant impact on the population during this period of human history, and doctors and people of other professions were considered to belong to the priestly class. The cause of many diseasesand pains was attributed to spirits and demons. The most common treatment method was surgical intervention. Diseases of the eyes, mouth, and teeth were frequent, and the cause of tooth decay was ascribedto the "tooth worm." Preserved written documents also reveal therapeutic modalities for treating periodontal diseases, which primarily involved rubbing the teeth with specific ointments made by mixing plants, resins, and spices. These ointments were used for toothaches and loosened teeth, and treatment would continue until bleeding occurred, which was considered a positive sign leading to recovery [16]. In ancient Egypt (3000 BC), medicine was based on religious beliefs, as was their culture, architecture and art. This has been proven by numerous writings on papyri. The Edwin Smith Papyrus is the oldest written

work on surgical treatment. It also contains the first anatomical and pathological descriptions of the human body, diagnosis, and treatment plans. The first descriptions of cauterization of pathological tissue and tumor removal are found in this papyrus. Descriptions of extracting loosened and intact teeth indicate that periodontal diseases were known to physicians of that era. In the therapy for diseased periodontal tissue, plant-based ointments were used to reduce discomfort and pain as well as to strengthen the tooth within the alveolus. These ointments were made from plants, honey and spices and applied by rubbing them into painful areas or near the teeth causing discomfort [2]. The Egyptian scribe Hesy-Ra, who lived during the time of the 3rd Egyptian dynasty, was the first dental physician in ancient Egypt. Among other things, his tomb bears the title "the greatest among those who deal with teeth." He is also credited with being the first person to recognize periodontal diseases [17]. Biological remains from the Phoenician period (2500 BC) indicate that this civilization was aware ofthe issues of loosened and even lost teeth. One of the methods they used for solving this problem is by binding the loosened teeth together with gold wires [3]. (Figure 1.)

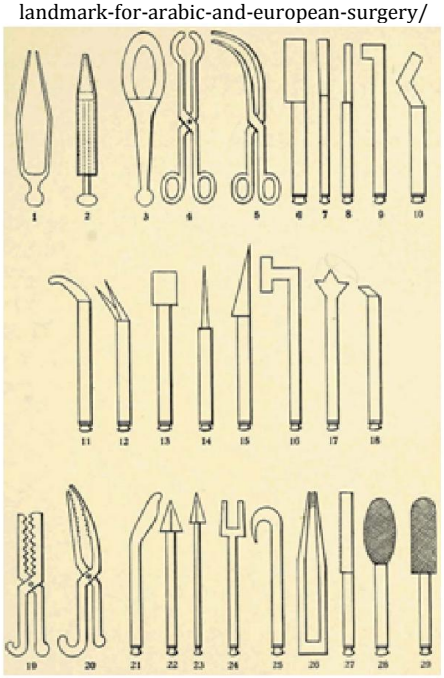
Figure 1. Golden wire ligature used as a therapeutic procedure in the treatment of tooth loosening
Source: https://mydesultoryblog.com/2018/10/when-it-comes-to-dentistry-be-glad-you-live-in-the-21st-century/



The most prominent figure in medicine in ancient Greece (750–320 BC) was Hippocrates (460–370 BC). He is still considered as the father of medicine, and his oath is taken in many countries after completing studies in medicine and relatedmedical fields. His contribution to modern medicine iswell known. Among other things, he recognized the importance of clinical observation and patient evaluation. He spoke aboutthe role of nature in the healing process and opposed therapeutic methods that could harm the patient. Hippocrates introduced the concept of disease prevention through a healthy diet and healthy environment. He described jaw dislocations and fractures as well as ulcers occurring in the mouth due to systemic diseases. One of the topics he dealt withwas the etiology of periodontal diseases, along with an explanation of tooth eruption. He attributed gum inflammation to the accumulation of hard deposits on teeth (i.e. tartar) and spleen diseases. In one patient with a spleen disease, he noted a swollen abdomen, acute pain and gums that separated from the teeth with an unpleasantsmell [18]. In addition to Hippocrates, Aristotle (384–322 BC) was a leading scientist, teacher and philosopher in ancient Greece. He researched the etiology and observed symptoms of oral diseases. Aristotle wrote about periodontal diseases and tooth decay, described the morphology of teeth and considered the importance of occlusion. He studied the harmful effects of sugar and sticky foods on teeth and periodontal tissues. As an example, he examined the impact of figs on periodontal health, pointing out their sweetness and sticky texture as causes of cavities and gum inflammation. Aristotle believed that deposits remained in periodontal tissues and between teeth, in areas where their removal was difficult [4]. In ancient Rome, physicians primarily focused on preventing periodontal diseases. During this period (10 BC–25 AD), oral hygiene was considered an important factor in public health. Many preserved documents mention the use of toothbrushes and their significance [11]. The first prominent physician was Aulus Cornelius Celsus (25 BC–10 AD). In his books, which contained all existing knowledge of medicine at that time, he described various treatments for periodontal diseases alongside the significance of prevention and oral hygiene. In one of his works, he wrote that bleeding gums should be treated with apple juice and vinegar. He also described using cauterization to remove enlarged gums and advised applying honey and

red wine to the area after the procedure [5]. The Islamic Golden Age, spanning from the 8th to 13th centuries AD, marked significant cultural, scientific, and technological advancements in the Islamic world. Among the notable figures of this era was Abulcasis, the most renowned physician of the western Caliphate. He described hemophilia, surgical removal of metastasized tumors and the use of cauterization to stop bleeding from damaged blood vessels. Abulcasisrealized that deposits and tartar on teeth were etiological factors in the development of periodontal diseases. He understood the importance of removing deposits from both the teeth and spaces beneath the gums, leading him to design and create a series of instruments for effectively removing these deposits. These instruments were the forerunners of modern dental scalers and curettes [15]. (Figure 2.)

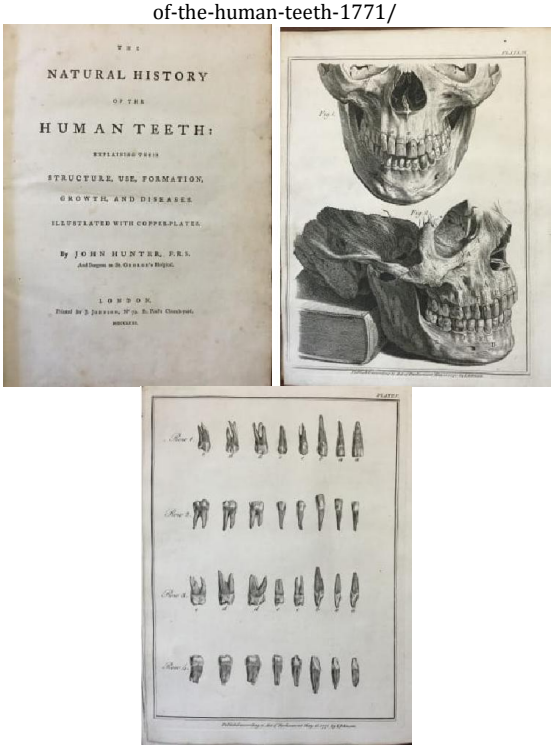
Figure 2. Sketches of periodontal instruments designed by Abulcasis
Source: https://medheritage.org/2023/03/01/abulcasis-a-landmark-for-arabic-and-european-surgery/



The Renaissance brought new momentum to art, culture, medicine and dentistry. Books and writings emerged, describing human anatomy more thoroughly than before, thanks to advancements in dissection methods [6]. The invention of the printing press enabled the production of books in greater numbers and the translation of medical texts contributed to the spreadingof medical knowledge. The pursuit of science and research became more accessible and many took advantage of this opportunity.The first printed book in the field of dentistry was published in 1530 under the title "Little Medicinal Book for All Kinds of Diseases and Infirmities of the Teeth" by ArtzneyBuchlein. It included descriptions of previously known information, therapeutic methods and the experiences of physicians from ancient Greece and Rome, as well as observations and knowledge from Arab physicians during the Islamic Golden Age. Several chapters of this book discussed diseases of the gingiva andperiodontal tissue [7]. Bartolomeo Eustachio (1500–1574), an Italian scientist, made significant contributions to medical science, particularly in human anatomy. Alongside detailed descriptions of tooth structure and pulp, he described oral diseases and offered treatment methods. His approach to treating periodontitis and periodontal disease was considered modern for that era. He advised the removal of dental tartar and excision of pathologically altered tissue [9]. Surgical procedures on oral tissues were the subject of his further research.AmbroiseParé (1510–1590) was the first to describe oral surgical and other procedures, earning recognition as the greatest surgeon of the Renaissance. He refined numerous surgical instruments for oral procedures and understood the importance of tartar in the etiology of gingival and periodontal diseases, emphasizing the necessity of its removal [8]. In the 18th century, two names stand out as particularly significant for the further development of periodontology: Pierre Fauchard and John Hunter. Fauchard, a French navy warsurgeon, was particularly interested in oral diseases affecting sailors aboard ships. The condition that caused the most trouble among sailors was scurvy. His skills became widely known and he soon gained fame. He successfully was treatingdental cavities, tartar issues and the removal of benign tumors in the oral cavity. Fauchardis notable for performing the first gingivectomyprocedure in 1742, a method for removing excess gingival tissue. In his books, he detailed the anatomy of the gingiva, changes in its structure and morphology, and epulides [15]. John Hunter, a British physician and scientist, whoanalyzed in detail and studied human anatomy. During his lifetime, he collected over 13,000 anatomical specimens, which he studied

and described in his publications. His manuscripts on head anatomy were supplemented with illustrations of jaw and facial bones, as well as primary and permanent teeth. Hunter also described the morphology of teeth with root canals and pulp tissue (Figure 3). As a surgeon, he believed that all diseases and changes in the bony and soft tissues of the oral cavity should be treated by surgeons. He tracked the progression of dental cavities, describing “white spot fields” on teeth that later advanced to brown areas representing decayed tooth tissue. Hunter also described "pockets" in the bone and their destructive effects, leading to tooth loosening [10].

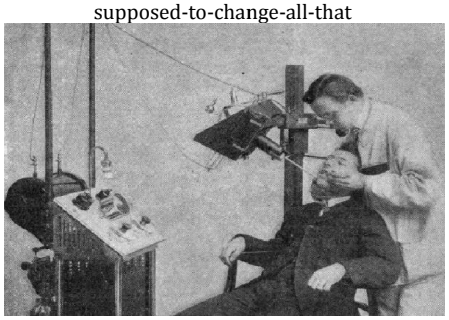
Figure 3. The book by John Hunter from the year 1771
Source: - https://www.rcseng.ac.uk/library-and-publications/library/blog/john-hunter-the-natural-history-of-the-human-teeth-1771/



The 19th century was a period of great medical discoveries and advancements. Research in pathology and microbiology, the discovery of anesthesia and X-rays revolutionized diagnostic concepts and improved the treatment of oral diseases. Germany and America took leading roles in clinical therapeutic procedures and research. John Riggs (1811–1855) was a prominent physician who focused on periodontal diseases and their treatment. His interest in gingiva and its diseases began early in his clinical career. Due to his success in diagnosis and treatment, as well as his decision to dedicate his clinical and scientific work entirelyto periodontal tissue diseases, Riggs is considered the first periodontistin history. His importance in understanding and treating periodontal diseases is highlighted by the fact that the disease now recognized as periodontitis was once called "Riggs' disease." [11]. Riggs opposed gingival surgical resection, a procedure widely used at the timeand insisted on removing pathological tissuein the subgingivalregion. He emphasized the importance of prevention and oral hygiene, particularly tooth brushing. Leonard Koecker (1785–1850), a dentist and researcher, also played a significant role in the development of periodontology in the 19th century. His observations and innovative ideas reshaped the understanding of periodontal diseases. By examining and following patients, he noticed that periodontal diseases begin with gingival inflammation, which he described as "slowly progressing." He identified hard deposits on teeth as a critical etiological factor in disease onset and emphasized the necessity of regular tooth brushing after meals. Koeckeralso addressed the concept of "focal infection," suggesting that teeth with poor prognosis and retaineddental roots should be extracted to prevent diseases in distant organs [21]. The 20th century saw continuous development in science and technology, partly influenced by the wars of this era. The pursuit of power and overcoming adversaries required the involvement of the brightest minds across all fields. The discovery of antibiotics led to successful treatments for numerous infectious diseases. The use of X-rays and magnetic resonance improved diagnostic accuracy and opened doors to innovative therapeutic procedures, such as organ transplants. Dentistry followed medical advancements, integrating technological and scientific achievements. Following military medicine, dentistry adopted X-rays as a diagnostic tool [12] (Figure 4). Machine-powered dental instruments began replacing manual ones, and dental chairs with reflectors were designed. Local anesthesia started being used during interventions. The discovery of fluoride's preventive role in cavity formation improved oral health and prevented dental and periodontal diseases. New periodontal

instruments were created to aid in diagnosing and treating periodontal diseases. The first periodontal probe, then called a "periodontometer", was designed, allowing better diagnosis of periodontal pockets and measurement of their depth. Measurements recorded during follow-ups enabled dentists to monitor treatment outcomes [13]. Both causal, non-surgical therapy and surgical procedures of the periodontal tissueunderwent itsrenaissance. Robert Newman and Leonard Widmanwere pivotal figures in the development of periodontal surgery during this period. Widman's flap procedure from 1918 marked a breakthrough in periodontal surgical advancements. This innovative method featured a trapezoidal flap shape with two vertical relaxation incisions, providing greater mobility and better access to pathological tissue [19]. A decade later, in 1931, Kirkland introduced a new approach called "subgingival open-field curettage." This procedure aimed to preserve healthy periodontal tissue and suppressinflammation, by removinginflamed and pathologically altered tissue [20]. Post-World War II, a new generation of scientists and clinicians took over the role from their predecessors. A world recovering from significant losses began focusing on innovative solutions and rebuilding society. Caring for people's needs became a societal norm. In the U.S., oral health improved continuously thanks to preventive measures introduced. Surgical procedures for the periodontal tissuebecame increasingly widespread, with investments made in new materials and instruments. A notable development in treating periodontal diseases was the local use of penicillin. Administered in lozenge form, its slow breakdown ensured prolonged concentration in the oral cavity. Indications included acute ulcerative gingivostomatitisand acute streptococcal tonsillitis. Penicillin was also used preventively before certain oral surgical interventions and in patients with systemic diseases. Later, penicillin became the drug of choice for intramuscular administration in patients with acute and necrotizing gingival changes [14].

Figure 4. The first commercial dental X-ray machine (1905)
Source: https://paleofuture.com/blog/2016/8/30/going-to-the-dentist-in-1909-was-a-nightmare-but-x-rays-were-supposed-to-change-all-that

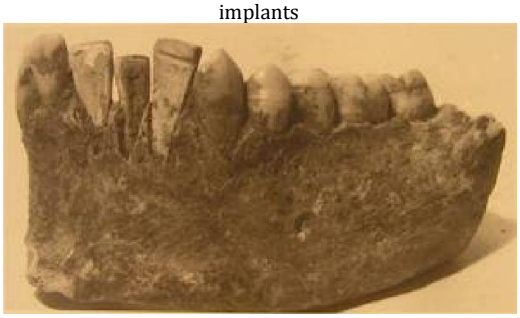


This period also marks the birth of a new dental discipline – implantology. The idea of replacing an extracted tooth with another tooth or material dates back to the earliest human civilizations (Figure 5). However, it was not until the 20th century that implantologybegan to develop as a science. A major breakthrough in implantologytherapy was the discovery by Swedish orthopedic surgeon Brånemark. During one surgical procedure, he used biocompatible titanium intraosseousimplants and later described the term "osseointegration," which became the foundation of modern implantology [22].

Today, the treatment of diseased periodontal tissuerequires a thorough approach, precise analyses, and consideration of all potential outcomes of the applied therapy. Periodontistsnot only treat diseases of the tooth-supporting structure but also assess their impact on the entire body. Periodontal diseases (periodontitis) can affect the health of many systems and organs, while on the other hand, numerous systemic diseases manifest their signsin the oral cavity.Modern periodontal surgery, besides modified and improved surgical procedures and implantology, strives for a regenerative and reconstructive approach to treatment. Prior to any intervention, emphasis is placed on a comprehensive examination, evaluation of theperiodontal tissue, and the planning of therapeutic procedures. For this purpose, numerous computer programs are now utilized, enabling clinicians to plan appropriate treatments and evaluatethe success of the applied therapy using three-dimensional models.Research in regenerative surgical procedures and biomaterials has led clinicians to aim to restore original lost periodontal tissues, thereby returning function and morphology to

the tooth-supporting structures. Stem cell research has increased the success rate of regenerative and reconstructive therapies for advanced periodontal diseases. All surgical procedures have been adapted to be minimally invasive, with a focus on preserving as much healthy periodontal tissue as possible [23].

Figure 5. Mandible from the Mayan period , with three implanted pieces of shell in a toothless space
Source: https://dreamdentalimplants.com/history-of-implants



CONCLUSION

Modern periodontology has little in common with the treatment methods practiced in past centuries. One of the few similarities is that periodontal diseases continue to be a challenge for humanity. Bleeding gums, pain, and tooth loosening are just some of the symptoms that drove earlier civilizations to search for solutions to this problem. For this reason, historical data is of great importance in understanding the development of periodontology. Today, periodontology has advanced significantly and encompasses a wide range of areas, starting from diagnosis and non-surgical therapy to occlusaltherapy, resectiveprocedures and the regeneration of hard and soft tissues of the tooth-supporting structures.

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DOCTOR SMILJA KOSTIĆ – LIFE AND LEGACY OF A FORGOTTEN HEROINE

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Abstract: Doctor Smilja Kostić was a pioneer in the field of medicine, making significant contributions, particularly in the areas of immunology and infectious diseases. One of her most important missions was combating infectious diseases, which were among the leading causes of mortality in Yugoslavia during the first half of the 20th century. During the Balkan Wars, at the age of seventeen, Smilja volunteered as a nurse at the Third Reserve Hospital in Belgrade. Her exceptional humanity and bravery during the Balkan Wars earned her the Cross of Mercy and the Bronze Medal of the Serbian Red Cross. She began her medical studies in 1915 in Lausanne, continued them in Montpellier, and graduated in 1919 in Strasbourg. After completing her studies, she returned to Belgrade, where she became the first female docent at the Faculty of Medicine in Belgrade. One of her most notable achievements was the introduction of the BCG vaccine to the country. For her outstanding contributions to the fight against tuberculosis, she was awarded the National Order of the Legion of Honor by France. In the post-war period, she remained unaffiliated with any ideological organization, which led to accusations of harboring a hostile attitude toward the socialist community. As a result, she was prematurely retired and dismissed from the faculty. Half a century after her dismissal and twenty years after her death, she was posthumously rehabilitated. Doctor Kostić was much more than a physician and scientist. She was a humanist, a leader, and a visionary whose influence transcended the boundaries of medicine.
Keywords: woman, physician, pioneer, achievements, vaccine, tuberculosis

INTRODUCTION

n the world, in the period after the Second World War, the BCG vaccine became widely accepted as a preventive measure against tuberculosis. Our country was among those that actively implemented this prevention, and the doctor who made an immeasurable contribution to its application in our region was awarded the Legion of Honour. The broader public only learned in 2020 that this doctor was Dr. Smilja Kostić [1,2].

Dr. Smilja Kostić was a pioneer in the field of medicine, with significant contributions, especially in immunology and infectious diseases. Born in Belgrade in the first half of the 20th century, Dr. Kostić devoted her life to advancing medical knowledge and healthcare, which made her one of the most respected figures in the scientific community.

One of her most important missions was the control of infectious diseases, which at that time were among the leading causes of mortality in Yugoslavia. With the development of science and technology, Dr. Kostić was among the first in our country to study the immune system and its responses to various pathogens. Her research and practical fieldwork significantly improved the understanding of methods for preventing and controlling the spread of infectious diseases, thereby helping to safeguard the health of the entire community.

In scientific research, Dr. Kostić was known for her innovative approach and great dedication. In addition to working with patients, she was actively involved in educating young medical professionals, believing that education was key to long-term improvement of the healthcare system. She also founded several centers for the study of immunological responses, where she worked on developing new therapeutic methods and disease prevention strategies [3,4,5].

Dr. Kostić's work remains highly significant today, as her discoveries and methodologies laid the foundation for further development of immunology and infectious disease medicine in Serbia and beyond. Her legacy lives on through generations of doctors and researchers who continue her mission, applying her findings and methods in the daily fight for human health. Through her dedication and passion for science, Dr. Smilja Kostić left a profound and lasting mark in medicine, and her work continues to inspire today.

EARLY CHILDHOOD AND EDUCATION

Dr. Smilja Kostić was born as Smilja Joksić in 1895 in Belgrade, to her father Momčilo Joksić, an adjutant of the Royal Guard, and her mother Staka Pačić. She was the second of seven children born to Momčilo and Staka. She was the great-granddaughter of Toma Vučić Perišić, a participant in the First and Second Serbian Uprisings and one of the most influential figures in Serbia in the second half of the 19th century. It is assumed that she inherited from her great-grandfather her persistence, determination, and skill in dealing with people, especially children.

Her schooling was neither easy nor continuous. She completed primary and secondary education in Belgrade and passed the so-called maturity exam in 1913. During the Balkan Wars (1912–1913), at the age of seventeen, Smilja voluntarily enlisted as a nurse at the Third Reserve Hospital in Belgrade. For her great humanity and bravery shown during the Balkan Wars, she was decorated with the Cross of Mercy and the Bronze Medal of the Serbian Red Cross.

She began medical studies in 1915 in Lausanne, during the difficult years of war. Her studies were interrupted once again when, at the beginning of the First World War, she volunteered to serve as a nurse in the Military Hospital in Kragujevac. She resumed her medical studies in Montpellier and graduated in 1919 in Strasbourg, where she began working at the Children's Clinic. In 1921, she defended her doctoral thesis and earned the degree of Doctor of Medicine.

FAMILY

During her studies, in 1919, she married her colleague Aleksandar Kostić. Their civil ceremony took place in Montpellier, and the religious wedding was held in Nice, at the Russian Orthodox Church. They had two children: a son, Ivan (Vanja), who died in 1942 as a fighter in the Ravna Gora movement, and Vojislav (Voki) Kostić, a well-known composer and gastronome. [3,6,7,8].

RETURN TO SERBIA AND PROFESSIONAL CAREER

After completing her studies, Smilja Kostić stayed for a short time in Strasbourg. In 1922, she returned to Belgrade with her husband, Prof. Dr. Aleksandar Kostić. The reason for their return was Aleksandar Kostić's appointment as a professor of histology at the newly established Faculty of Medicine in Belgrade. When the Histological Institute of the Faculty of Medicine in Belgrade acquired its own premises in the 1920s, Dr. Smilja Kostić became the first assistant at that department.

She held this position for a short time, as in 1924 she became an assistant at the newly established Children's Clinic, where she founded the Children's Dispensary and later became head of the service. From that period, her pioneering work in pediatric medicine began, with far-reaching consequences. The Children's Dispensary became a center for preventive medicine and child health care, laying the foundation for modern pediatric practice in Serbia.

Dr. Smilja Kostić applied contemporary global methods in scientific research, teaching, and medical practice. Fluent in German and French, she not only wrote original works but also translated professional literature. She published over 120 professional and scientific papers as well as popular pediatric articles in Serbian and French, in both domestic and international journals.

During her career, in addition to her dedication to pediatric education, she worked as chief physician of the Children's Clinic outpatient department and as a physician in the Infant Advisory Clinic, where she guided mothers on newborn care and nutrition. She authored the first manuals in Serbia for clinical laboratory diagnostics. Besides research on cardiovascular diseases, antibiotic applications, and metabolic and nutritional disorders in children, by the late 1920s her professional focus shifted to a major public health problem—childhood tuberculosis—and its prevention.

She was appointed assistant professor at the Department of Pediatrics in 1939, becoming the first female assistant professor at

the Faculty of Medicine. At the beginning of World War II in 1941, she was removed from the faculty for refusing to sign the "Appeal to the Serbian People," but two years later she was reinstated and continued in her position until the end of the war. She was promoted to associate professor at the Department of Pediatrics in 1948, and at the same time appointed head of the main department of the Children's Clinic in Belgrade. [2,9,10].

CONTRIBUTION TO TUBERCULOSIS PREVENTION AND INTRODUCTION OF THE BCG VACCINE

Dr. Smilja Kostić was a pioneer in the fight against childhood tuberculosis in Yugoslavia. Her dedication to implementing preventive measures and the use of the BCG vaccine represented a crucial step in reducing the incidence and mortality of this dangerous disease.

In the first half of the 20th century, tuberculosis was one of the deadliest diseases, especially in poor and war-affected regions like Yugoslavia. Children were among the most vulnerable groups, often falling ill due to poor living conditions, inadequate nutrition, and lack of medical care. At that time, the country had one of the highest child mortality rates under the age of five caused by tuberculosis. Dr. Kostić recognized the seriousness of the problem and dedicated her work to combating tuberculosis through prevention and education. As the head of the Children's Dispensary, she initiated programs for early detection and treatment of tuberculosis in children, a revolutionary approach at the time.

One of her most important achievements was the introduction of the BCG vaccine in Yugoslavia. This vaccine, developed in France in the 1920s, became a fundamental tool in the global fight against tuberculosis. Its introduction in Yugoslavia faced challenges due to lack of resources, trained personnel, and awareness of the importance of immunization. Dr. Kostić tirelessly advocated for the vaccine, using scientific evidence and international experience to convince authorities and the health system of its effectiveness. She organized educational campaigns to raise awareness among doctors and the general population, which led to the gradual acceptance of vaccination. Her efforts culminated in the beginning of mass immunization of children against tuberculosis, which soon resulted in a significant reduction in the number of cases.

Dr. Kostić not only introduced the vaccine but also worked on implementing comprehensive preventive measures. Through her work at the Children's Dispensary, she developed programs including regular health check-ups, early diagnosis, and treatment for children with tuberculosis or at risk of infection. She focused particularly on educating mothers and families about the importance of hygiene, nutrition, and healthy living conditions in disease prevention. Her programs also included training of medical personnel to ensure proper care for children nationwide. Her results, demonstrating the practical value and safety of the vaccine, were recognized by the Pasteur Institute and children's clinics in Paris and Stockholm as a significant scientific contribution.

For her exceptional contribution to the fight against tuberculosis, Dr. Smilja Kostić received a highly prestigious award. In 1952, she was decorated with the National Order of the Legion of Honor by France, one of the most esteemed recognitions at the time. The French President also presented her with a brooch in the shape of the initials "BCG" set with diamonds, honoring her pioneering work in promoting the vaccine. Her husband, Prof. Dr. Aleksandar Kostić, had received the same award 12 years earlier for his contributions to science. At the time of her decoration, there was only one other couple in the world with the same distinction: Marie and Pierre Curie [2,8,11].

PROFESSIONAL CHALLENGES

During her successful career, she faced numerous obstacles and difficulties. In her home country, some of her attitudes and actions were not well regarded. Because she did not participate in any ideological organization in the post-war period, she was labeled as a professor showing a hostile stance toward the socialist community. Her ideological and political positions did not align with the society's expectations, where intellectuals, particularly university professors, were required to show cooperation and loyalty. After a well-organized political campaign, she was officially morally and politically discredited and retired on June 1, 1954, effectively being removed from the faculty [9,12].

LATER LIFE AND LEGACY

Smilja and Aleksandar Kostić were inseparable from their youth. They lived together for 70 years, reaching old age side by side. After being removed from the Faculty of Medicine, Dr. Smilja Kostić dedicated the last three decades of her life to private practice and assisted her husband in writing and research endeavors. She passed away on June 5, 1981, in Belgrade, in the family apartment at Dositejeva 1. Thanks to their son, Vokie Kostić, the couple rests together in the Alley of Distinguished Citizens at the New Cemetery in Belgrade. According to their wish, the memorial plaque reads:

"INSEPARABLE IN LIFE, INSEPARABLE AFTER DEATH."

Dr. Smilja Kostić was morally rehabilitated in 2001, becoming the only woman among 31 professors removed from the faculty after the war to receive this recognition. In 2018, as part of the renovated Grocka cultural-historical complex, her legacy was publicly showcased in the exhibition gallery of the Ilija Garašanin Library, where, alongside her husband, she found her place in life and posthumously [2].

CONCLUSION

The life and work of Dr. Smilja Kostić exemplify unwavering dedication to science, medicine, and humanity. As the first female assistant professor at the Faculty of Medicine, University of Belgrade, she broke barriers at a time when women's roles in science were limited. Her pioneering achievements in pediatrics and public health left an indelible mark on the medical history of Serbia.

Dr. Kostić not only treated patients, but also educated and inspired her students, colleagues, and community to recognize the importance of health and prevention. Although her work was internationally recognized, she faced many challenges. Her selfless dedication and perseverance were not always appreciated in the political and social context of her time, resulting in the injustice of her removal from the Faculty of Medicine.

Her posthumous rehabilitation and recognition confirm the lasting significance of her work. Dr. Smilja Kostić was more than a doctor and scientist; she was a humanist, leader, and visionary, whose impact transcended medicine. Her legacy lives on through the health of generations of children, improved public health systems, and the inspiration she provides to new generations of physicians. Through her commitment and persistence, she became a symbol of the fight for science, justice, and a better life for all. Her name remains recorded in history as a synonym for integrity, expertise, and love for humanity..

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TOOTH DECORATION THROUGH HISTORY: BETWEEN AESTHETICS, IDENTITY, AND RITUAL

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Abstract: Tooth decoration has been practiced by various civilizations worldwide, often extending beyond aesthetics into the realms of identity, ritual, and spirituality. From gemstone inlays among the Maya, to tooth blackening in Japan, and contemporary trends such as grillz, dental ornamentation represents a rich spectrum of cultural expression. This paper examines the historical development, symbolic meanings, and cultural significance of tooth decoration, with particular attention to its implications for modern dental practice

Keywords: tooth decoration, dental ornamentation, cultural practices, symbolism, history of dentistry, ritual tooth modification, aesthetic dentistry

INTRODUCTION

Body modification for aesthetic, social, and religious purposes is as old as civilization itself [1]. In nearly all parts of the world, the human need to express identity, affiliation, or status has been reflected through bodily interventions, including those involving teeth [2]. Teeth, as prominent and easily visible facial elements, have played a key role in many ritualistic and decorative practices. Their transformation—whether through pigmentation, the insertion of precious metals and gemstones, filing into various shapes, or even deliberate extraction—has been observed across different cultures. Such practices can be found among the civilizations of Mesoamerica, especially the Maya, in ancient Egyptian tombs, and throughout East and Southeast Asia—in Japan, China, and the Philippines [3,4]. Each of these cultures developed unique techniques and aesthetic norms that reflected their cosmologies, social hierarchies, and concepts of beauty [5].

This paper focuses on geographically and chronologically diverse examples of dental adornment throughout history, aiming to identify and analyze the cultural meanings, techniques, and materials used in these practices.

ANCIENT CIVILIZATIONS

One of the most striking forms of dental modification in ancient Mesoamerican civilization was the practice of tooth decoration among the Maya. This practice carried deep symbolic meanings and was closely tied to aesthetics, social status, and religious beliefs. The Maya often drilled small holes into their front teeth using rotary tools, into which they embedded semi-precious stones such as jade, turquoise, or hematite. Jade, symbolizing immortality, power, and a connection with deities, was especially reserved for members of the elite [6].

In addition to gemstone inlays, tooth reshaping through filing was also common, producing pointed, T-shaped, or notched forms. These procedures were typically performed during youth, likely as part of initiation rites, adding to their social and spiritual significance. Some communities even practiced staining or blackening of the teeth with special substances, which was considered a sign of purity and attractiveness. These sophisticated interventions demonstrate the high level of technical skill possessed by the ancient Maya, as well as the deeply rooted importance of oral aesthetics in their culture and daily life [7].

Among Andean peoples such as the Inca, symbolic tooth filing and the use of red or black pigments were practiced. Certain colors were believed to offer protection from evil spirits or aid in ritual transformation. Although the Incas did not embed decorations as the Maya did, the symbolic importance of teeth was evident in many ceremonial contexts.

In contrast to the Maya, there is no direct evidence of gemstone inlays or deliberate aesthetic reshaping of teeth among the ancient Egyptians. However, archaeological findings

indicate that Egyptians placed great emphasis on oral hygiene and aesthetics. They also developed early forms of dental procedures, including the stabilization of loose teeth with gold wire, which some interpret as an early form of dental prosthetics. In the tombs of wealthy individuals, gold prostheses and inlays have been found, likely serving both aesthetic and symbolic purposes, as well as indicating social status. In some cases, teeth were wired together post-mortem as part of funerary preparations aimed at preserving bodily "wholeness" for the afterlife [8].

CHINA AND EAST ASIA

In East Asian cultures, dominant dental practices involved staining, blackening, and surface modification. In ancient China, particularly during the Han and Tang dynasties, tooth coloring was practiced in rural areas and especially among ethnic minorities. Blackening of teeth carried multiple meanings—from protection against evil spirits, to expressions of purity, and visual harmony aligned with Taoist principles of yin and yang [9].

This practice later spread to Vietnam. The most notable example in Japan was ohaguro, an aesthetic ritual of tooth blackening practiced from the Heian period (9th century) until the late 19th century. Ohaguro symbolized maturity, femininity, fidelity, and sophistication, and at times was also a component of samurai culture. The process involved a mixture of iron filings, vinegar, and plant-based pigments [10,11].

Unlike their Asian neighbors, ancient Filipino civilizations developed highly complex forms of dental ornamentation using gold restorations. Archaeological discoveries such as the famous Bolinao skull reveal that individuals during the precolonial era had gold inlays and engravings on their front teeth—serving both as status symbols and spiritual protection [12,13]. Some rulers and nobles even had diamond-encrusted restorations, highlighting an advanced level of dental technology and aesthetic standards in precolonial societies [14].

During the Vedic period in India, dental hygiene played a significant role in spiritual and health practices. While direct modifications of the teeth were uncommon, records exist of Ayurvedic preparations used for whitening and strengthening teeth, associated with the concept of spiritual and physical purity. Later, among some aristocratic classes, the use of gold inlays and ornamental dental elements was also documented [15].

AFRICA

Africa is a continent with some of the most diverse and enduring traditions of body modification, where dental decoration and reshaping have played an important role in many communities. The most common dental interventions involved deliberate tooth extraction, filing, sharpening, and pigmentation [16].

The Mangbetu people (Democratic Republic of Congo) are known for skull elongation, but also for the aesthetic shaping of teeth. Young girls and boys would have their front teeth sharpened into a triangular shape, which represented an ideal of beauty and group identity. These modifications were part of initiation rituals and symbolized physical readiness and aesthetic maturity [17].

Among the Yaka and Teke peoples (Congo and Angola), as well as the Makonde (Mozambique), tooth filing into sharp points was a ritual act of sexual maturity and also served as a means of intimidating enemies during times of war. Teeth were considered "windows to the soul," and their transformation and protection had a spiritual dimension. This process was painful and often performed during adolescence [18].

The practice of coloring teeth and tattooing the lips with black pigments among Fulani women in northern Mali and Niger is deeply rooted in their culture and aesthetics. This tradition, known as Tchoodi or tunpungalle, includes tattooing the gums, lips, and chin with natural pigments, often derived from plant sources such as ash and resins. The goal of these modifications is to highlight the whiteness of the teeth, which is seen as a symbol of purity, beauty, and spiritual balance. These practices are often performed during ceremonies and weddings, representing a rite of passage into adulthood and symbolizing courage and community belonging [19].

Members of the Beti and Fang peoples (Cameroon and Gabon) practiced the removal of upper front teeth as part of initiation into adulthood. It was believed that this act liberated the individual from childhood and opened a spiritual channel for communication with ancestors. The absence of teeth was not seen as a handicap, but as a sign of honor and bravery [20].

In East African tribes such as the Dinka and Nuer in Sudan, the removal of lower incisors has been practiced for centuries. It was believed that extracting these teeth made it easier to ingest food and medicine during illness, but it also had symbolic meaning—it marked the transition from childhood to adulthood [21].

Although more widely known for other forms of body modification (e.g., ear stretching), some Maasai warriors (Kenya and Tanzania) had their teeth removed during initiation as a symbol of sacrifice and masculinity. They also practiced forms of traditional dental therapy, often involving symbolic "treatment" of pain through pigmentation and rituals [22].

EUROPE

Dental decoration in Europe followed a distinct developmental path, differing from the traditions of other continents, and was mostly closely associated with social status, aesthetics, and the technological capabilities of the time.

Archaeological findings suggest that as early as ancient Rome, wealthier classes used dental inlays made of gold or bone. While the focus was on functionality and restoration, there were also aesthetic elements of adornment [23]. Concern for white teeth and oral hygiene was part of cultural norms, and well-preserved smiles were seen as a sign of refinement.

During the Middle Ages, the dominance of Christian dogma diminished the importance of bodily aesthetics, but among the European nobility in Italy and France, gold and silver teeth were status symbols. These modifications had no health-related purpose and were part of courtly luxury and personal aesthetics [24]. Simultaneously, in rural areas of Europe, such as Scotland and Ireland, coloring teeth with plant-based pigments—especially using tree bark—was common among women. Darker teeth were seen as a sign of modesty and piety.

The period from the 17th to the 19th century marked a revolution in dental prosthetics. In England and France, sophisticated prosthodontics were developed, and the aesthetic of a white smile became a dominant ideal linked to cleanliness and morality. This period is known for the phenomenon of "Waterloo teeth"—natural teeth collected from the battlefield after the Battle of Waterloo and used to make dentures for the wealthy [25].

During the Spanish Inquisition (1478–1834), the Catholic Church strictly forbade "unnatural adornment" of the body, including the teeth, deeming such practices heretical and contrary to religious norms. Although there is no concrete evidence of engravings on teeth as secret religious symbols, it is known that some clandestine religious orders used discreet body markings as signs of affiliation and spiritual devotion [26].

Among Slavic peoples, particularly in Ukraine and Russia, archaeological findings show practices of decorating teeth with metal wires and gold threads, often for ceremonial purposes. Warriors wore inlays as signs of courage and tribal affiliation, while aristocrats developed early forms of dental prosthetics using gold and mother-of-pearl [27].

CONTEMPORARY SUBCULTURES AND ARTISTIC PRACTICE

Modern technologies have made dental decoration less invasive, more accessible, and safer for patients. In the 20th and 21st centuries, European artistic and musical subcultures (e.g., punk, goth, and hip-hop communities) popularized dental adornment through piercings, decorative tooth covers (grillz), and laser engravings. In Germany and France, artists have emerged who engrave images and messages onto dental veneers, merging dentistry with art [28].

Tooth gems remain among the most popular aesthetic dental accessories. A recent trend emphasizes minimalism—tiny zirconia stones, diamonds, or shapes such as stars, moons, and similar symbols are commonly used [29]. The emergence of nano-tattoos for teeth introduces a novelty in aesthetic dentistry. These temporary tattoos are applied directly to the enamel and last from several days to a week. They are safe for use and often chosen for special occasions [30].

In urban styles, there is growing popularity of gold and metallic caps that cover a single tooth—most often a canine or lateral incisor. Contemporary versions of these accessories are sophisticated, often featuring matte finishes or rose gold coloring.

Tooth decorations that glow under UV light have become a trend among festivalgoers and attendees of nighttime events. These adornments are easy to apply and remove, do not damage the teeth, and come in various colors and shapes [31].

Although grillz have long been present in popular culture, modern examples are far

more advanced—they are crafted using intraoral scanning and 3D design, often incorporating engravings, symbols, or initials, and are made from various metal alloys [32].

One of the most modern expressions in the field of dental aesthetics is the concept of geometric porcelain veneers—lovja. These veneers, known for their unique surface texture featuring multifaceted geometric shapes (dentagons), are made from highly aesthetic ceramics and are characterized by precision and individualized design [33].

CONCLUSION

Dental decoration throughout history reflects a complex connection between aesthetics, identity, and ritual. From ancient civilizations, where teeth symbolized status and religious beliefs, to modern trends that merge personal expression with technology, this phenomenon has evolved over time. Today, thanks to new dental technologies, tooth decoration is becoming more accessible and less invasive, allowing for greater personalization. Although the symbolic and social aspects have changed, dental decoration remains an important form of identification, with the potential to further evolve in response to the needs of contemporary society.

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EPIGENETIC THEORIES OF BRUCE LIPTON AND THEIR SCIENTIFIC EVALUATION

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Summary: The paper analyzes Bruce Lipton's epigenetic theories and their scientific evaluation. Lipton, a former professor of cellular biology, challenges traditional genetic determinism by claiming that beliefs and perceptions can directly influence gene expression. In his book The Biology of Belief, he emphasizes that the cell membrane, rather than DNA, functions as the "brain" of the cell, mediating between the environment and genetic expression. While the scientific community acknowledges some fundamental insights in epigenetics, it expresses significant reservations about Lipton's claims regarding the direct impact of thoughts on DNA, highlighting methodological shortcomings and the problematic application of quantum physics to biological systems. Despite the criticisms, his theories raise important questions about mind–body interactions, with potential implications for the development of integrative medicine. Lipton's most notable contribution lies in challenging existing paradigms and fostering dialogue between different approaches to health that go beyond the strictly mechanistic model of the human body.
Key words: epigenetics, Bruce Lipton, biology of belief, cell membrane

INTRODUCTION

In recent decades, scientific understanding of genetics has changed significantly, leading to a reevaluation of traditional concepts in molecular biology. Epigenetics, which studies changes in gene expression without altering the DNA sequence, has opened new theories about the interaction between organisms and their environment. In this context, the work of Bruce Lipton stands out as one of the most influential yet controversial contributions to the contemporary understanding of genetic determinism. As a former professor of cellular biology, Lipton challenges the dominant dogma of molecular biology, which asserts that genetic material exclusively determines the structure and function of living beings. He proposes a model in which the cell membrane plays a key role in the interaction between the organism and its environment, suggesting that our beliefs and perceptions can directly influence biochemical processes and gene expression. This paper analyzes Lipton's theories, critically examines them, and explores their implications for the future of medicine, emphasizing the tension between mechanistic and holistic approaches to health and disease.

REVOLUTION IN GENE UNDERSTANDING: LIPTON'S EPIGENETIC TURN

Bruce Lipton is a biologist and author known for his controversial ideas on epigenetics and consciousness. His books, such as The Biology of Belief, explore the connection between thought and biological processes. Lipton asserts that our beliefs and perceptions can influence our genes and cellular biology, an idea that diverges from traditional molecular biology. Before his career as an author, he worked as a professor of cellular biology at a medical school. His portrait is shown in the following figure..

Figure 1. Bruce Lipton, molecular biologist
Source: https://www.edurazvoj.com/da-li-geni-odredjuju-sudbinu-deteta-ili-je-za-to-odgovorna-okolina-epigenetika-daje-odgovor/

Over the past decades, the understanding of genetics has undergone significant shifts, leading to a reevaluation of traditional paradigms in molecular biology. In this context, the work of Bruce Lipton represents one of the most controversial contributions to contemporary understandings of genetic determinism. As a former professor of cell biology at the Stanford University School of Medicine and a researcher at the University of Wisconsin Medical School, Lipton developed theories challenging the dominant central dogma of molecular biology, which posits that genetic material solely dictates the structure and function of living organisms [1].

Lipton's epigenetic paradigm shift begins with his radical departure from genetic determinism, which has dominated biological sciences since the discovery of DNA. Rather than accepting genes as the primary controllers of biological processes, he emphasizes the cell membrane as the key interface between the organism and its environment, suggesting that signals from the external environment are the main mechanism regulating genetic expression. In his book The Biology of Belief, Lipton argues that DNA is not the "brain" of the cell; instead, the cell membrane responds to environmental stimuli and transmits signals that induce epigenetic changes [1].

A central element of Lipton's epigenetic shift is the thesis that our perceptions and beliefs can directly influence biochemical processes in the body, altering the way our genes are expressed. Drawing on research in psychoneuroimmunology, Lipton claims that psychological factors such as stress and emotions can trigger biological changes through epigenetic mechanisms [2]. This approach challenges the classical biomedical model, suggesting that the mind and psychological processes can modify matter at a fundamental level.

Lipton's research in stem cell biology further supports his theories on environmental influence on cellular behavior. Through experiments, he demonstrated that identical stem cells, when exposed to different environments, could develop into different cell types despite having the same genetic material [3]. These findings underscore the flexibility of genetic expression and the importance of epigenetics as a mechanism for adaptation to external conditions.

The revolutionary aspect of Lipton's ideas lies in his holistic approach, linking diverse scientific disciplines. His integration of quantum physics, cell biology, psychology, and spirituality represents an attempt to create a unified framework for understanding life, which has generated skepticism in some scientific circles [4]. Nonetheless, such interdisciplinarity opens new perspectives and raises questions that conventional approaches may overlook.

Lipton also highlights the evolutionary significance of epigenetic mechanisms, suggesting that they enable faster adaptation to changing conditions than classical genetic selection. His approach proposes a neo-Darwinian synthesis that incorporates random mutations and natural selection while asserting that organisms possess sophisticated mechanisms for actively adapting to their environment through epigenetic modifications [5].

The epigenetic shift advocated by Lipton has profound implications for medicine and therapy. Accepting that beliefs and perceptions have biochemical consequences opens the door for complementary treatment approaches that integrate psychological and spiritual components [3]. Although his theories are controversial, Lipton's contribution to understanding epigenetics cannot be ignored, inspiring new generations of scientists to challenge established dogmas.

Lipton's epigenetic paradigm calls for a shift in the way we understand life, encouraging reflection on our potential for self-healing and responsibility for our health [6]. His theory opens new horizons for research that may lead to a more comprehensive understanding of complex biological systems and their interactions with the environment.

MIND OVER MATTER: CENTRAL PREMISES OF LIPTON'S THE BIOLOGY OF BELIEF

In his book The Biology of Belief (2005), Bruce Lipton presents a revolutionary thesis asserting that our beliefs and perceptions can directly influence genetic expression and cellular physiology. This perspective challenges the traditional biomedical model of DNA determinism, emphasizing that the cell membrane functions as the "brain" of the cell, mediating between the external environment and internal biochemical processes. Lipton

argues that environmental signals—including those generated by our thoughts and beliefs—can significantly impact gene expression [1].

Lipton develops the concept that beliefs act as energetic filters shaping our biochemical reality. His research suggests that the state of mind can modify cellular behavior through complex signal transduction systems. Based on experiments with cell cultures, Lipton proposes that positive beliefs can enhance health, while negative mental patterns may contribute to disease development [7]. One key aspect of Lipton's theory is the reconstruction of the relationship between consciousness and biology. He rejects the mechanistic model of the human body, replacing it with a model in which thoughts and beliefs are fundamental determinants of health. Lipton asserts that we can consciously reprogram our DNA by changing beliefs, introducing the concept of "epigenetic engineering" to modify harmful subconscious beliefs, often formed during childhood [2].

In critiquing traditional genetics, Lipton relies on findings from the Human Genome Project, which revealed a smaller number of genes than previously expected. This suggests that genetic material alone cannot fully explain the complexity of human physiology. Instead, he emphasizes the role of epigenetic mechanisms, which affect gene expression without altering the DNA sequence, highlighting the environment as a critical factor in manifesting genetic potential [7].

Lipton links individual epigenetic processes to broader social and evolutionary considerations, arguing that humanity is undergoing an evolutionary shift in which collective consciousness may overcome biological limitations. In the book Spontaneous Evolution, co-authored with Steve Bhaerman, they explore how collective beliefs shape not only individual health but also the evolutionary trajectory of the human species. For Lipton, understanding the connection between mind and biology is essential for improving human health and developing a new model of medicine that recognizes the power of the mind over matter. [2].

UNDER THE SCIENTIFIC LENS: A CRITICAL ANALYSIS OF LIPTON'S CLAIMS

The scientific community has taken a nuanced stance toward the epigenetic theories proposed by Bruce Lipton. While many experts acknowledge some of the foundational insights he offers, they simultaneously express serious reservations about his broader conclusions. Lipton's assertions regarding the power of consciousness to directly influence gene expression through the concept of a "new biology" significantly exceed what is currently supported by contemporary research. Molecular biologists, in particular, are critical of his simplified interpretation of cellular mechanisms and the overstated role of the cell membrane as the "true brain" of the cell, which departs markedly from accepted models of cell biology [8].

A fundamental issue with Lipton's theories lies in his methodological approach. Conventional science requires rigorous hypothesis testing, statistical validation, and reproducibility of results, whereas Lipton's claims often rely on anecdotal evidence and selective interpretation of scientific literature. Critiques are particularly directed at his conclusion that thoughts and beliefs can directly reprogram our genes, which oversimplifies the complex epigenetic mechanisms documented in empirical research [9].

Although the impact of stress and other psychological factors on physiology is real and well-established through psychoneuroimmunology, Lipton extends these findings far beyond empirically validated boundaries. Scientific studies indicate that psychological factors can influence biochemical pathways that may lead to epigenetic changes, but they do not support claims that consciousness can directly and voluntarily alter DNA without intermediary biological processes. This gap between established mechanisms and Lipton's assertions represents the primary reason for scientific skepticism [10].

Moreover, a significant portion of the criticism focuses on Lipton's selective use of quantum physics to support his biological theories. Applying quantum principles to macroscopic biological systems constitutes a problematic simplification that overlooks the scale and complexity differences between quantum and cellular systems. Physicists and biologists generally agree that while quantum effects may play a role in certain biological processes, such as photosynthesis or magnetoreception, Lipton's extension of these phenomena to explain the power of

consciousness via quantum mechanics is not empirically substantiated [11].

It is important to note that critical perspectives on Lipton's theories do not imply a wholesale rejection of epigenetics or psychoneuroimmunological connections. On the contrary, these fields represent exciting areas of research with a growing body of evidence. However, the scientific community insists on a precise distinction between established facts and speculative hypotheses. The current scientific consensus acknowledges the complex interactions between mind and body but remains skeptical of simplified explanations that fail to adequately account for the intricacy of biological systems [12]. Critiques of Lipton's theories highlight a broader challenge in science—balancing openness to novel ideas with the maintenance of rigorous standards of evidence. While some aspects of his theories may inspire new research questions, scientific evaluation requires that such hypotheses undergo systematic investigation before they are widely accepted. This epistemological caution does not represent a rejection of innovative thinking but rather reflects a scientific methodology committed to robust and reproducible findings. [13].

AT THE EDGE OF PARADIGMS: IMPLICATIONS OF LIPTON'S THEORIES FOR THE FUTURE OF MEDICINE

Contemporary medicine stands at a crossroads between the mechanistic model and holistic approaches. Bruce Lipton's epigenetic theories occupy an increasingly prominent, albeit controversial, position within this context. Lipton asserts that cells respond to environmental perception rather than solely to genetic predetermination, opening new understandings of disease mechanisms and treatment strategies [1].

The central question raised is what primarily determines health—genes or environment. While traditional genetics emphasizes genetic determinism, Lipton's interpretation of epigenetics focuses on perception and beliefs as key modulators of biological processes. This approach has the potential to transform medical practice from a system centered on symptoms to one that considers mental states and environmental influences on physiological processes [8].

Research in psychoneuroimmunology and neuroendocrinology provides empirical support for some aspects of Lipton's theories, particularly regarding stress and the immune system. Chronic stress has been shown to influence gene expression through epigenetic modifications, further highlighting the importance of psychological factors in biological functioning [14].

However, the scientific community remains cautious about Lipton's broader conclusions.

Integrative medicine, which combines conventional medical practices with complementary approaches such as stress management and psychological interventions, may represent the first practical application of Lipton's principles. This approach acknowledges the role of psychological factors in physical health, although it does not fully endorse Lipton's theories [15].

The placebo effect further illustrates the relevance of Lipton's ideas. Once considered merely a methodological confound, it is increasingly recognized as a phenomenon demonstrating the power of belief to modify physiological processes. Lipton's theories on perception may help explain this effect, although definitive mechanisms linking beliefs to epigenetic modifications remain unestablished [16].

Bioethical challenges also arise from Lipton's perspective. Accepting that perception can influence health raises questions of responsibility—who is accountable for illness and recovery? While this may empower patients, it also carries the risk of attributing blame to individuals for conditions beyond their control [17]. Medical education will need to evolve in light of Lipton's theories. Current curricula emphasize molecular aspects, whereas integrating epigenetic principles could enrich future physicians' understanding of the mind-body-environment interplay [18].

Critiquing Lipton's theories does not imply rejecting the mind-body connection; rather, it calls for more precise research. The concept that psychological factors influence physiology through epigenetic mechanisms represents a legitimate field of investigation [19]. Lipton's contribution may lie less in providing concrete mechanisms and more in challenging existing paradigms and stimulating research that transcends current boundaries.

Regardless of whether his theories withstand the test of time, his ability to provoke dialogue between different approaches to health represents a meaningful contribution to the evolution of medicine. [20].

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DR. ĐORĐE JOANOVIĆ, THE FIRST SERBIAN ONCOLOGIST

Dijana Piljić

PRIMARY HEALTH CARE CENTER "NOVI BEČEJ", NOVI BEČEJ

Summary: Đorđe Joanović was born on June 16, 1871, in Vienna, to father Hariton and mother Marija. The Joanović family originates from the village of Beodra. He interrupted his scientific work and career at the University of Vienna and, after the end of World War I, moved to Belgrade out of a patriotic desire to help war-torn Serbia. Dr. Đorđe Joanović was a world-renowned scientist and one of the pioneers in laboratory research on oncological diseases and the epidemiology of carcinogenesis. He was the founder of the University of Belgrade Faculty of Medicine, the initiator of the establishment of the Institute of Pathology in Belgrade, founder and president of the Yugoslav Society for the Study and Suppression of Cancer, and dean of the Faculty of Medicine in Belgrade. Professor Dr. Đorđe Joanović was a corresponding member of the Royal Serbian Academy of Sciences, an honorary member of Matica Srpska in Novi Sad, permanent delegate of the Kingdom of Yugoslavia to the International Office of Public Hygiene in Paris, member of the German and Czech Oncology Committees for Cancer Control, member of the editorial boards of numerous medical journals, president of the Serbian Medical Society, president of the Yugoslav Medical Society, lecturer for the military medical service, and member of the Sanitary Council of the Army of the Kingdom of Yugoslavia. He was also president of the fund for assisting poor students, member of the Committee of the International Institute for Geographical Pathology, editor of the Serbian Archive of General Medicine, and representative of the Kingdom of Yugoslavia in the All-Slavic Medical Union. The Institute of Pathology in Belgrade, the elementary school in Novi Miloševo, and the General Hospital in Zrenjanin bear the name “Dr. Đorđe Joanović.”

Keywords: Dr. Đorđe Joanović, Institute of Pathology, Belgrade – Oncology, Epidemiology of Carcinogenesis

INTRODUCTION

Professor Dr. Đorđe Joanović is recognized as the founder of oncology and experimental pathology in Serbia. He holds the distinction of being the first Serbian oncologist. At his time, Dr. Joanović attained the highest academic rank and position among Serbs worldwide, with a particular focus on experimental oncology, pathology, oncological pathology, and pathological morphology. His contributions to the development of medicine, especially oncology in Serbia, are of immeasurable significance.

Professor Dr. Joanović’s scientific work in experimental pathology and immunopathology was pioneering even on a global scale, earning him an impressive international reputation. He dedicated himself to experiments on oncological diseases and studied the epidemiology of carcinogenesis. With special interest, he conducted studies on pathological changes in tissues across various diseases, always giving careful and meticulous attention to pathological anatomy and histology.

Professor Joanović earned a reputation as one of the most important scientists globally, demonstrating through his work and actions the true meaning of patriotism and devotion to one’s country.

THE JOANOVIĆ FAMILY

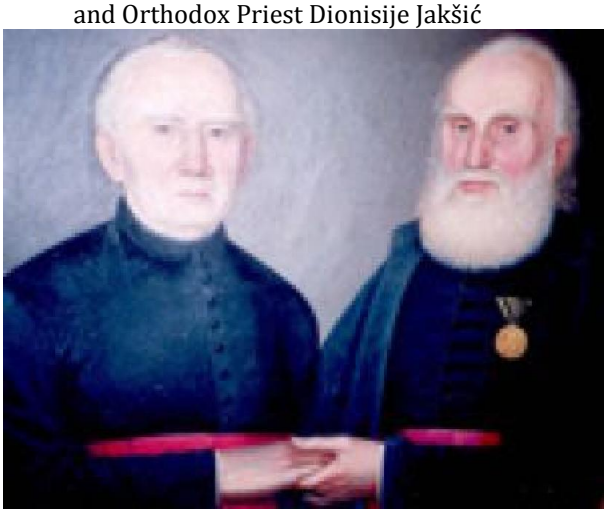
Dr. Đorđe Joanović was born in Vienna to his mother Marija and father Harinton. His mother, Marija, née Vlahović, was originally from Veliki Bečkerek. His father, Harinton, was born in Beodra, which today is a settlement within the village of Novo Miloševo.

The Joanović family traces its ancestry to the southern Serbian province of Kosovo and Metohija. Đorđe Joanović’s grandfather, Aksentije Joanović, was an Orthodox priest and parish priest of the Orthodox church community in Beodra. Aksentije was a close friend of Dionisije Jakšić, an Orthodox priest from the neighboring village of Karlova. Dionisije Jakšić

was the father of the renowned Serbian poet and painter Đura Jakšić.

The sons of Aksentije and Dionisije, Harinton Joanović and Đura Jakšić, grew up together and became close childhood friends.

Figure 1. Orthodox Priest Aksentije Joanović and Orthodox Priest Dionisije Jakšić



Aksentije sent his son Harinton to study in Vienna, where Harinton graduated in law and remained to live and work. The entire Joanović family visited their ancestral village of Beodra at least once a year. Harinton Joanović (1824–1884), a lawyer and senator, served as manager for the "Austrian Railway Magnate" Baron Sine in Vienna. In Vienna, Harinton married his wife Marija in 1868, and they had two sons: Đorđe and Simeon Joanović.

The Joanović household upheld family traditions, and both Đorđe and Simeon were raised in the patriarchal spirit of old Serbian customs. For the godfather of his sons' baptisms, Harinton chose Baron Sine of Vienna. Đorđe's father, Harinton, and mother, Marija, were remembered as generous benefactors. In 1852, the Serbian painter and poet Đura Jakšić came to Vienna to continue his art studies, and his childhood friendship with Harinton's family from Beodra continued in Vienna. Đura Jakšić was provided with accommodation and meals in the Joanović household for an entire year.

Both of Harinton's sons, Đorđe and Simeon, completed higher education in Vienna. Đorđe Joanović became a professor at the Medical Faculty in Vienna, while Simeon Joanović served as a consul of the Kingdom of Serbia in Vienna. Simeon Joanović (1868–1934) was the Austrian vice-consul in Belgrade (1885–1897), and from 1901, the civil commissioner of Austria-Hungary in Pljevlja, where Đorđe Joanović, as an Austro-Hungarian subject, was appointed in 1897 as the head of the newly built military hospital at Stažica in Pljevlja. After retiring, Simeon Joanović authored the multi-volume book "Novopazarski Sandžak 1878–1900."

Simeon Joanović lived for extended periods in Belgrade and Zurich. Afterward, he returned with his wife Ana to the family estate in Beodra, where he was often visited by Dr. Đorđe Joanović. The Joanović family was respected and esteemed in their native village. Locals affectionately called Professor Dr. Đorđe Joanović "Doctor Đoka".

Figure 2. Professor Dr. Đorđe Joanović

BIOGRAPHY OF DOCTOR ĐORĐE JOANOVIĆ

Education of Dr. Đorđe Joanović

Đorđe Joanović completed his primary education in Vienna and subsequently enrolled in the highly esteemed Vienna Gymnasium "Kaiser und König," where he graduated in 1889. He then entered the Medical Faculty in Vienna, graduating in 1895. Immediately after completing his studies, he was employed at the same faculty as an assistant from 1895 to 1899 in the Institute of Pathological Histology and Bacteriology. He later moved as an assistant to the Institute of General and Experimental Pathology, where he worked from 1899 to 1904.

During this period, he worked under the renowned pathologist Professor Paltauf (1858–1924), a student of Pasteur and Koch. Joanović was appointed as a docent in 1904. He became an associate professor of general and

experimental pathology at the Vienna Medical Faculty in 1910 and a full professor in 1919. At that time, the rector of the University of Vienna was the famous pathologist and academician Carl von Rokitansky (1804–1878). The main rivals of the Vienna Medical School worldwide were Berlin, Paris, London, and to some extent St. Petersburg.

Dr. Joanović led the Department of General Medical Pathology in Vienna, conducting advanced experiments in prestigious laboratories of the Institute of Pathology. For the next twenty-five years, he remained a dedicated and respected collaborator. During this time, he held the highest academic rank and position among Serbs globally, with a particular focus on experimental oncology, pathology, oncological pathology, and pathological morphology.

Arrival of Dr. Joanović in Serbia

After the end of World War I, Dr. Milan Jovanović-Batut and Dr. Vojislav Subotić invited Dr. Đorđe Joanović to come from Vienna to Serbia and contribute his expertise and organizational skills to the establishment of the Medical Faculty in Belgrade. Dr. Joanović accepted the invitation without hesitation. At the first session held on February 20, 1920, the inaugural assembly of professors of the Medical Faculty in Belgrade took place. Dr. Milan Jovanović-Batut was elected dean, and Dr. Đorđe Joanović was appointed full professor of general pathology.

At that time, Dr. Joanović made the decision to leave his scientific work in prestigious Viennese laboratories and his university career in Vienna. He moved to Belgrade on May 6, 1920, with a strong desire to contribute to the development of medicine in war-torn Serbia. He faced significant organizational challenges and obstacles, including some unjust opposition from certain individuals.

Figure 3. The first eight professors of the Medical Faculty in Belgrade; from left to right: Dr. Miloš Bogdanović, Dr. Richard Burian, Dr. Vladan Đorđević, Dr. Pavle Popović, Dr. Đorđe Joanović, Dr. Milan Jovanović-Batut, Dr. Milivoje Kostić, and Dr. Slobodan Kostić.

In 1924, Professor Richard Paltauf passed away in Vienna. About twenty esteemed German pathologists applied to fill his university chair, but the Faculty Council of the Medical Faculty in Vienna chose to invite Dr. Đorđe Joanović to return and occupy Paltauf's position. This was a significant recognition of Dr. Joanović's expertise and knowledge. Despite difficult working conditions in Belgrade, he declined the offer, wishing to complete the projects he had begun in Serbia.

After the founding of the Medical Faculty in Belgrade, Dr. Joanović worked on establishing the Oncology Service of the Kingdom of Serbs, Croats, and Slovenes and provided the initiative and detailed conceptual plans for the Institute of Pathology in Belgrade. The construction took three years, during which he actively participated, giving concrete advice. Based on his designs and ideas, the new Institute of Pathology building was completed and officially opened on April 22, 1926. At the opening, Dr. Joanović delivered the inaugural speech. The Institute quickly became a regional center for experimental pathology. His collaborators included Ksenofon Šahović (1898–1956), Dimitrije Tihomirov, Marija Višnjić, Živojin Ignjačev, and others. Dr. Joanović spent entire days in the laboratories, even converting his office into his living quarters.

Almost every day at 8 a.m., he attended autopsies; at noon, he delivered lectures, and in his free time—usually in the afternoons—he worked with colleagues on diagnosing

histological samples. Twice a week, at the end of practical histology exercises, he personally explained the data for students using projections of histological specimens. Patients with neoplasms often requested consultations with him, which he always granted, providing comprehensive advice and frequently written recommendations.

In 1926, he became a corresponding member of the Serbian Royal Academy of Sciences. Dr. Joanović dedicated his life entirely to medical science and the fight against cancer. On September 27, 1927, he founded the Yugoslav Society for the Study and Suppression of Cancer, with K. Šahović as general secretary. At that time, this society was the fourth of its kind in the world, after Vienna (1910), Washington (1917), and Paris (1920). He represented Serbia and Yugoslavia at numerous medical congresses in Europe and the United States.

Professor Dr. Đorđe Joanović was the only dean of the Medical Faculty to be elected four times (1923/24, 1925/26, 1927/28, 1928/29). He also founded the Association of Yugoslav Physicians, serving as its honorary and lifelong president. He was president of the Serbian Medical Society and a close friend of medical students, elected as lifelong honorary president of the Yugoslav Medical Students' Union. In 1928, he was awarded the Order of Saint Sava, Second Class. He was the initiator of the idea to establish the Institute for Oncology and Radiology in Belgrade, which was completed in the autumn of 1939 under the patronage of Her Majesty Queen Maria, with Prince Pavle Karađorđević as the main donor.

Dr. Joanović brought his vast medical knowledge and university reputation from Vienna. He was known for his expertise, precision, and meticulousness. Privately, he loved music and art and was an accomplished violinist. He had a quiet and modest nature that charmed and won over his interlocutors. Students and colleagues deeply respected, valued, and loved him, appreciating his support and advocacy for student autonomy at the university.

He was especially beloved as a professor who sought to understand and help students with their problems. However, his solidarity with students seeking university autonomy displeased city authorities, leading to significant difficulties. In 1929, during the proclamation of the Obznana and the introduction of the January dictatorship, the regime harshly targeted political opponents, including students. Although not politically active, he supported students and workers. According to some reports, on January 27, 1932, he had a heated dispute with Prime Minister General Petar Živković, which ended with the general slapping him.

Dr. Đorđe Joanović never married. When asked why, he answered similarly to Nikola Tesla: "When I began experiments in pathology, I realized that science requires a whole person." His brother Simeon Joanović was married to Ana but had no children, so the Joanović family line unfortunately ended..

TRAGIC END OF PROFESSOR DR. ĐORĐE JOANOVIĆ

The epilogue of Dr. Joanović's life was tragic. In 1932, while organizing the annual St. Sava Students' Ball, medical students informed him that King Alexander I of Serbia was welcome to attend, but not his Prime Minister, General Petar Živković. General Živković summoned Dr. Joanović to his office, where they engaged in a bitter argument. Humiliated, Dr. Joanović stormed out and returned to his Institute of Pathology, to his room. On the morning of January 28, 1932, he was found hanged from a window latch, with his body lying in his armchair. A maid discovered the lifeless body and immediately called her husband, who worked as a janitor in the Institute building. He removed Dr. Joanović's body from the rope. Professor of Pathology Dr. Marija Višnjić-Frajnd reported that a large amount of ashes was found in the fireplace that morning, indicating that he had burned his personal files. The unexpected death of Professor Dr. Đorđe Joanović caused sorrow and surprise. The circumstances of his death remain unclear, and many suspect that he may have been murdered. His death provoked outrage among Belgrade intellectuals, as many immediately doubted that the professor had taken his own life.

The Medical Faculty Council decided to hold a brief memorial service in the amphitheater of the Institute of Pathology in Belgrade, while a religious ceremony would be conducted in his native village of Beodra. His remains were transported by train, accompanied by a large number of students, friends, and colleagues. Belgrade had never before witnessed

such a massive and solemn procession to the railway station for a funeral. Several hundred students, mainly from the Medical Faculty, attended the burial, along with many prominent figures from the public life of the Kingdom of Yugoslavia. Dr. Joanović was interred at Beodra Cemetery on January 29, 1932. Although his death was suspected to be a suicide, Serbian Patriarch Varnava authorized a funeral service for the esteemed deceased.

On February 28, 1932, a politically motivated article titled “The Case of Professor Joanović” was published by his opponents, Dr. Svetozar Moačanin and Dr. Dušan Petrović, claiming: "He came to Serbia to create, and therefore his principled views on the faculty morally and scientifically diverged diametrically from those of his opponents. Joanović came to teach students, not to seek titles, high salaries, or benefits from Belgrade sanatoriums and other political or financial advantages."

The article was met with condemnation from many contemporary intellectuals, students, and ordinary citizens. Dr. Dimitrije Tihomirov, assistant professor of general pathology and pathological anatomy at the University of Belgrade, wrote in Glasnik za staleška i zdravstvena pitanja (February 15, 1932):

"With feelings of profound and deep sorrow over this sudden, immense, and irreparable loss for the Medical Faculty and the Kingdom of Yugoslavia, I will attempt to give but a faint picture and brief characterization of the late teacher, who was simultaneously a Yugoslav and European scholar of great stature." Dr. Joanović’s international reputation was evident from a letter of condolence from the International Office of Public Hygiene in Paris, addressed to the Minister of Social Policy and Public Health: "The news of Professor Joanović’s death has caused us deep sorrow, for he was among the most highly esteemed scholars, and in his lectures, he was always most attentively listened to, despite being personally very modest."

Today, only words of admiration and respect remain for this great scientist and human being. Pharmacist Stevan Vukov from Zrenjanin, who has studied Dr. Joanović’s life and work, commented: "For a time, I was closer to the conclusion that Dr. Joanović was the victim of a crime than that he committed suicide. Those who take their own lives are denied a church funeral, yet he was buried with a priest present, according to all Serbian Orthodox Church customs. Over time, understanding more about Joanović’s noble character and high moral principles, I became more inclined to believe that he ended his own life as an act of moral superiority, refusing to compromise his principles." — Politika, May 11, 2020.

After his death, a “Fund” was established in his name for the best annual topics in experimental pathology. On December 10, 2007, on the occasion of marking 80 years of the fight against cancer in Serbia, Professor Dr. Đorđe Joanović was posthumously awarded the Golden Medal of the Serbian Society for the Fight Against Cancer, presented to Academician V. Kanjuh..

SCIENTIFIC CONTRIBUTIONS OF PROFESSOR DR. ĐORĐE JOANOVIĆ

Contribution to Medicine and Oncology

Professor Dr. Đorđe Joanović’s contribution to medicine, particularly oncology in Serbia, is immeasurable. His scientific work in experimental pathology and immunopathology was pioneering on a global scale, earning him an impressive international reputation. He authored 58 significant scientific papers and collaborated with two of the most prestigious medical journals in Europe. The foundation of his work had been laid during his time in Vienna. He devoted himself to experiments on oncological diseases and studied the epidemiology of carcinogenesis. Professor Joanović paid special attention to pathological changes in tissues caused by various diseases, with a particular focus on pathological anatomy and histology.

The modern Vienna school of pathology was developed by Professor Richard Paltauf together with his closest collaborators, Professor Karl Stanberg and Professor Đorđe Joanović. Joanović’s first scientific paper, published in 1899, addressed the origin and significance of plasma cells during pathological processes. From this early work, he turned to oncology, then a relatively new field, and began conducting experiments in advanced Viennese laboratories on oncological pathology.

Work in Experimental Oncology and Oncological Pathology

Dr. Đorđe Joanović was the first Serbian oncologist-scientist, starting his work in

oncology in 1920. At that time, thanks to the Berlin pathologist Rudolf Virchow (1821–1902), the basic principles of oncological pathology were already recognized.

Professor Joanović focused extensively on the study of cancer, especially on factors influencing the growth of experimental carcinomas and sarcomas, including: diet, chronic anemia, various alkaloids, and extirpation of endocrine glands. He believed that predisposition, local changes, and general metabolic disturbances were critical in cancer development.

He investigated the pathological morphology of tumors, including bronchogenic carcinoma, cystic neck tumors, atheroma calcification and ossification, tumor development through irritation, and the multicentric origin of tumors within an organ. He studied tumor growth in vivo and in vitro using tumor tissue cultures.

Professor Joanović observed that castration and splenectomy promoted tumor growth, while rice also stimulated growth. Certain toxic substances, including small doses of morphine, cocaine, and quinine, as well as toluidinediamine intoxication, slowed tumor growth. He explored the immunological aspects of cancer therapy using fermentative extracts from tumor tissues of the same patients. Lymphectasia, plasma cell accumulation, and connective tissue proliferation with sequestration of carcinoma cells were indicative of tumor regression in experimental animals.

Considering that erysipelas infection could destroy skin carcinoma, he also experimented with bee venom in cancer therapy. His publications provide extensive reviews of experimental cancer research and the effects of radium. Dr. Joanović's overall insight into cancer etiology and pathogenesis offered authentic and relevant information about the state of oncology in his era.

Work in Experimental Pathology and Pathological Morphology

Dr. Đorđe Joanović is regarded as the founder of oncology and experimental pathology in Serbia. He also researched staining of microorganisms in pathological tissues, studied liver pathology, and authored significant scientific papers in this area. His work on liver disease included studies on the pathogenesis of jaundice, recognized by the Belgian Royal Medical Academy.

His experimental studies demonstrated that jaundice induced by toluidinediamine disappeared after splenectomy, providing a foundation for therapeutic splenectomy in hemolytic jaundice. Of particular interest are his experimental studies on the liver and fat metabolism. Dr. Joanović's work in experimental pathology and pathological morphology covered various areas, including tetanus prophylaxis, anaphylactic shock, transplantation problems, and nutritional pathology. His scientific contributions laid the groundwork for modern pathology and oncology in Serbia and earned him international recognition as a pioneering researcher.

Discoveries on Autoimmunity and Final Assessment of Professor Dr. Đorđe Joanović

Discoveries on Autoaggression (Autoimmunity)

Professor Dr. Đorđe Joanović observed that soldiers with healed head and brain injuries from firearms sometimes suffered severe headaches and even died. Autopsies revealed multiple areas of softened brain tissue both near and distant from the healed injury site. Similar results were obtained in his experiments on white rats subjected to mechanical head injuries.

From these observations in humans and animals, he concluded that brain degradation products stimulate the immune system to produce antibodies not only against these products but also against similar substances in healthy brain cells, leading to widespread brain softening.

Joanović attempted to apply this original etiopathogenetic concept to therapy for superficial skin carcinoma and tuberculous granulomas in animals by injecting disintegrated carcinoma or granulomatous tissue (or digested Koch bacilli) under the skin of the same subjects. He also treated dermatoses, such as Trichophiton tonsurans infections, using disassociated products, and psoriasis using scarammas (scrapings). This method of treatment using coagulation products was successfully applied in psoriasis therapy. He presented these findings at the International Office for Hygiene in Paris in 1929, after which

his institute further developed work using this approach.

Professor Joanović was a pioneer in identifying autoaggression processes in medicine. This discovery of autoaggression represents one of his most important scientific achievements, though it remains little known both in Serbia and abroad. He first presented his findings in 1920 before the Vienna Medical Society and published two papers that year in the Viennese clinical journal Vochenschrift: "Effect of Brain and Bacterial Products Obtained by Enzymatic Destruction", "New Views on the Origin and Therapy of Certain Diseases". Through his results in experimental oncology and the discovery of autoaggression in medicine, Dr. Joanović gained a reputation as one of the most significant scientists internationally. In recognition, the Belgian Royal Academy awarded him a prize in 1903 for his scientific work.

Additionally, Professor Joanović conducted experimental studies on tuberculosis, the effects of radioactive substances on pathological organ changes, and non-surgical treatment methods for cataracts.

CONCLUSION

Professor Dr. Đorđe Joanović served for many years as head of the Institute of General Pathological Anatomy in Vienna. He was widely recognized as an exceptionally skilled specialist in pathological-anatomical diagnostics, a figure of great authority, trust, and esteem.

His contributions to medicine and especially oncology in Serbia are of immeasurable significance. His work in experimental pathology and immunopathology was pioneering even by global standards, earning him an impressive international reputation. Joanović possessed equal mastery of both the morphological and physiopathological aspects of his discipline, combined with a rare ability for acute observation, enabling him to reach correct conclusions quickly.

Professor Joanović devoted his entire life selflessly to science, spending full days at the Institute of Pathology. He was always approachable, remarkably attentive, and treated his students with fatherly care, explaining their errors patiently and supporting them, both morally and sometimes materially. His teaching extended beyond lectures and exercises; he used every opportunity to instruct and guide younger generations, exemplifying gentlemanly conduct, deep trust, and responsiveness.

In his interactions, Joanović demanded mutual trust and consensus. He was accessible to all students, physicians, and citizens. His kindness was well known to anyone who spoke with him or sought his counsel.

Professor Dr. Joanović was not only a great scientist but also a man of noble moral character. His imposing presence, calm demeanor, and composed behavior created the impression of a serene, steady, and approachable individual. By refusing to compromise on principles contrary to his values, he demonstrated moral superiority.

He was a man of rare virtues, devoted entirely to science, medical practice, and the dignity of his nation. A true patriot, he dedicated himself to the advancement of knowledge, the care of patients, and the service of his country, leaving a legacy of scientific brilliance and human nobility..

Figure 4. Primary School "Đorđe Joanović" Novo Miloševo, Beodrad

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A Review Article covers a systematically addressed specific medical problem, in which the author made some contribution, visible on the basis of self-citations. **Integral parts of the paper are: a) introduction**- (the aim of the review paper as the last paragraph of the introduction); **b) the text of the review of literatureonthe problem , with subtitles**; **c) conclusion; d) literature**. The review article is usually commissioned by the Editorial Board, but non-commissioned manuscripts are also considered. Contact the Editorial Board before writing a review article. Text length can be up to 5000 words (18 pages).

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Articles in the history of medicine and health culture shed light on certain aspects of medical practice in the past. Text length can be up to 2500 words (6 pages). These and the articles statedbelow do not have a prescribed structure, such as original papers, case reports, and review articles. Short contributions from the field of medical practice (diagnostics, therapy, remarks, suggestions and opinions on methodological problems, etc.) are published, too, as well as presentations from various medical meetings, symposia and congresses in the country and abroad, book reviews and articles from foreign journals up to 1000 words, 1-2 tables or images, up to 5 references (up to 3 pages of text). Editorial letters have up to 400 words, or 250 words if they contain comments on published articles. By order of the editorial board, or in agreement with the editorial board, works of didactic character are published.

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AUTHORSHIP. All persons listed as authors of the work should qualify for authorship. Each author should have participated sufficiently in the work on the manuscript to be able to take responsibility for the entire text and the results presented in the work. Authorship is based solely on: making a significant contribution to the concept of the work, obtaining results or analyzing and interpreting the results; the planning of the manuscript or its critical revision of considerable intellectual importance; the final refinement of the print version of the manuscript. Authors should attach a description of the contributions individually for each co-author within the Submission Letter form. Financing, collecting data or generally overseeing a research team cannot by itself justify authorship. All other contributors who are not the authors of the manuscript should be listed onthe

acknowledgementpage, with a description of their contribution to the work, with written consent, of course.

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ABBREVIATIONS. Use only when necessary, for very long names of chemical compounds, that is, abbreviations that are already recognizable (standard abbreviations, such as DNA, AIDS, HIV, ATP). For each abbreviation, the full term should be stated when first quoted, unless it is a standard unit of measure. Do not use abbreviations in the title. Avoid using abbreviations in the abstract, but if necessary, explain each abbreviation when first referenced in the text.

ACKNOWLEDGEMENTS. List all contributors who contributed to the creation of the work but did not meet the criteria for authorship, such as those providing technical assistance, writing assistance, or managing a department that provides general support. Financial and material assistance, in the form of sponsorships, scholarships, gifts, equipment, medicines and more, should also be listed

MANUSCRIPT PREPARATION

The text of the paper contains first and foremost the title of the paper, in the following lines: full names of the authors and all co-authors; the name, place and address of the institutions from which the author and co-authors come (in parentheses, associate the names of the authors); possibleacknowledgementfor help with elaborationofthe paper;

It is obligatory to submit:

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-**first and last name, year of birth of the author and all co-authors**;

-**full address, telephone and fax numbers, as well as the author's e-mail for correspondence**.

The following is a SUMMARY (Abstract), up to 300 words is best. A summary cannot have footnotes, tables, images, or references. A summary of **the original papers** should include: Introduction (state the objective in the last sentence), **Material and methods, Results and Conclusions**. Write each of the segments listed at the beginning of the sentence in bold. Provide the most important results (numerical values) of the statistical analysis and the levelofsignificance. The conclusion must not be general, but must be directly linked to the results of the work. **For case reports, the summary** should have the following parts: **Introduction** (state the objective in the last sentence), **Case report, Conclusion**. For other types of papers the summary has no specific structure.

The summary must not contain any claims that are not contained in the text of the article. It must be written in such a way that even an educated nonexpert can understand the content of the article. After the summary, write 3 to 8 keywords. The words in the title should not be repeated and the keywords should be relevant or descriptive and in accordance with MESH rules (available at https://www.nlm.nih.gov/mesh).

The next part of all the papers is an **INTRODUCTION** (with a subtitle of the same name), which must be brief, with a brief overview of the literature on the problem in question, and with a clear statement of **the purpose of the article** in a separate paragraph at the end of the introduction.

MATERIALS AND METHODS (with the same subtitle) must contain sufficient information to enable other researchers to repeat similar research without further information. Patient names and medical history numbers should not be used nor other details to help identify patients. The names of the apparatuses, software and statistical methods used must be indicated.

Show the **results** (with the subtitle of the same name in BOLD) clearly and concisely. You should not display the same data both in tables and charts.

DISCUSSION (with the subtitle of the same name) should discuss the interpretation of the results, their meaning in comparison with other, similar research and in accordance with the hypotheses of the research. The results already written should not be repeated.

CONCLUSION (with the subtitle of the same name) should be given in a separate chapter.

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Journal articles

Standard journal article:

Gao SR, McGarry M, Ferrier TL, Pallante B, Gasparrini B, Fletcher JR, et al. Effect of cell confluence on production of cloned mice using an inbred embryonic stem cell line. Biol Reprod. 2003; 68 (2): 595-603.

Organization as author:

WHO collaborative study team on the role of breastfeeding on the prevention of infant mortality. Effect of breastfeeding on infant and child mortality due to infectious diseases in less developed countries: a pooled analysis. Lancet. 2000; 355: 451–5.

No authors listed:
Coffe drinking and cancer of the pancreas [editorial]. BMJ. 1981; 283 628.

A volume with a supplement:
Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig heart anaphylaxis. Pharmacol Res Commun. 1988; 20 Suppl 5: 75–8.

Books and other monographs

The author is a person (s):
Carlson BM. Human embryology and developmental biology. 3rd ed. St. Louis: Mosby; 2004.

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Brown AM, Stubbs DW, editors. Medical physiology. New York: Wiley; 1983.

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Blaxter PS, Farnsworth TP. Social health and class inequalities. In: Carter C, Peel JR, editors. Equalities and inequalities in health. 2nd ed. London: Academic Press; 1976. p. 165–78.

Meeting announcements: Harris AH, editor. Economics and Health: 1997: Proceedings of the 19th Australian Conference of Health Economists; 1997 Sep 13-14; Sydney, Australia. Kensington, N.S.W.: School of Health Services Management, University of New South Wales; 1998.

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Anderson JC. Current status of chorion villus biopsy. In: Tudenhope D, Chenoweth J, editors. Proceedings of the 4th Congress of the Australian Perinatal Society; 1986: Brisbane, Queensland: Australian Perinatal Society; 1987. p. 190-6.

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Cairns RB. Infrared spectroscopy studies of solid oxygen. Dissertation. Berkley, California: University of California, 1965.

Electronic material

Article in aninternetmagazine:
Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs. 2002; 102 (6). Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002-Nov-15; 100 (10): 3828-31. Epub 2002 Jul 5.

CD-ROM:
Anderson SC, Poulsen KB. Anderson's Electronic Atlas of Hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Online monograph:
Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

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Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

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American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2screens]. Available from: http: //www.ama-assn.org/ama/pub/category/1736.html

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