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„Medicinska istraživanja“ je recenzirani naučni časopis Medicinskog fakulteta Univerziteta u Beogradu, koji je prvi put objavljen 1971 godine, dakle, pre tačno pola veka. Časopis „Medicinska istraživanja“ je prvenstveno posvećen publikovanju rezultata naučno-istraživačkog rada nastavnika i saradnika Medicinskog fakulteta, kao i drugih naučnika u oblasti biomedicinskih istraživanja. Časopis „Medicinska istraživanja“ objavljuje naučne radove na engleskom jeziku uz sažetak na srpskom jeziku i ima usvojenu politiku otvorenog pristupa (open access). Izlazi tri puta godišnje, dok se u četvrtoj svesci objavljuju sažeci izlaganja na simpozijumu „Stremljenja i novine u medicini“. U časopisu „Medicinska istraživanja“ se objavljuju originalni i prethodno neobjavljeni radovi: a. originalni radovi u kojima se prvi put objavljuju rezultati sopstvenih istraživanja; b. pregledni radovi (Review), kritički i originalno prikazani (sa autocitatima); c. prikazi bolesnika i slučajeva (Case report).
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GLAVNI I ODGOVORNI UREDNIK
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REVIEW

The concept and importance of ideal cardiovascular health

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Summary

Cardiovascular diseases (CVD) are the leading cause of death worldwide. In 2019, 17.9 million people died from CVD which accounts for 32% of all deaths globally. The burden of CVD in a given population is related to the cardiovascular health (CVH) of that particular population. In 2010, The American Heart Association initiated a new concept of ideal CVH focused on two groups, health behavior components (smoking, body mass index, physical activity, and diet) and health components (total cholesterol, blood glucose, and blood pressure). Ideal CVH is defined as the simultaneous presence of seven ideal CVH components or "Life's Simple 7" (non-smoking, body mass index <25 kg/m^2, level of physical activity up to the achievement of the set goal, diet in accordance with national recommendations, blood pressure <120/80 mm Hg, total cholesterol <200 mg/dL and blood glucose level <100 mg/dL) in the absence of clinically manifested CVD. The prevalence of ideal CVH is low worldwide and is less than 1% in the USA, China and Spain, and the lowest values (0.02% and 0,1%) were recorded in the Republic of Srpska, Bosnia and Herzegovina and Serbia, respectively. The ideal CVH is inversely related to the incidence and mortality rates of CVD, as well as the overall mortality rates. People with a greater number of ideal CVH metrics (5, 6, and 7) are significantly less likely to suffer from CVD, ischemic heart disease and stroke, and have lower mortality from CVD, as well as lower total mortality compared to people without any or with only one ideal component of CVH. The status of CVH in the population is essential for predicting the risk of morbidity and mortality from CVD, which can be reduced by improving both health/biological and behavioral components of CVH.

Keywords: ideal cardiovascular health, cardiovascular diseases, components of cardiovascular health, Life's Simple 7, prevalence, mortality

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INTRODUCTION

Cardiovascular diseases (CVD) are the leading cause of death globally and the main obstacle to sustainable human development (1). According to the estimates of the Global Burden of Disease Study 2015, which provides integrated data on incidence, prevalence and mortality, CVD are the main cause of years of life lost in all world areas (2).

Approximately 17.9 million people die from CVD each year, which accounts for 32% of all deaths worldwide. 85% of all deaths due to CVD are caused by heart attack and stroke (3).

In the USA, Australia and developed European countries, there has been a downward trend in CVD mortality rates in recent decades, primarily owing to positive changes in risk factors, which are responsible for about two-thirds of the decline in mortality rates, and successful evidence-based therapy accounting for one third of the drop in mortality rates (3-4). The lowest mortality rates from CVD were recorded in France, Israel, Spain, Denmark, the Netherlands, Norway, Switzerland and Great Britain, where standardized mortality rates were less than 350 per 100,000 men and less than 250 per 100,000 women (5).

However, despite the decline in CVD mortality in the developed world, CVD remains the most significant cause of death in developing countries (low- and middle-income countries), where over three quarters of all CVD deaths occur (3-4). Standardized mortality rates from CVD in European countries were highest in men in Ukraine, Belarus, Kyrgyzstan, and Russia (over 1,400 per 100,000), and in women in Kyrgyzstan, Moldova, and Ukraine (over 1,000 per 100,000) (5).

In Serbia as an upper middle income country CVD are responsible for 54% of all causes of death (6), and the standardized mortality rates from CVD in 2013 were 991 per 100,000 men and 836 per 100,000 women (5).

In addition to having the greatest contribution to mortality in the developed and developing countries, CVD also contribute significantly to the morbidity of the inhabitants of those countries. In 2015 there were approximately 422.7 million people with CVD worldwide (2).

Although CVD morbidity can be represented by several different indicators, including incidence rates, prevalence, as well as hospital discharge rates, the main limitation for the interpretation of CVD trends in European countries is precisely the lack of quality and comparable data on CVD and ischemic heart disease (IHD) morbidity (4). Hospital discharge rates for CVD show wide variation between European countries and do not reflect the same patterns as mortality rates in the same countries. They do not provide real data on the incidence of CVD, and differences between countries may arise, not only due to real differences in the CVD incidence, but also due to differences in the hospitalization regulations, the organization of health care and its efficiency, the way of coding, as well as the sudden death rates from CVD without hospitalization (7).

The extent of the CVD problem can also be expressed as the burden on the population by analyzing aggregate indicators (summary measures) like Years of Lost Life (YLL) and Disability Adjusted Life Years (DALY), i.e. the sum of years of life lost due to premature death and years lived with disability (8).

CVD are responsible for 11.8% of total DALYs; the main disease in the group is IHD (5.2%) followed by cerebrovascular disease (4.1%) (8).

In 2010, 4,282 DALYs (per 100,000) were lost due to CVD, mostly due to IHD (1,884 lost years per 100,000) and cerebrovascular disease (1,484 lost years per 100,000). IHD and cerebrovascular disease together were responsible for the premature death of 12.9 million people in 2010 (9).

The burden of CVD in a given population is related to the cardiovascular health (CVH) of that population (10). In 2010, the American Heart Association (AHA) introduced a novel concept of an ideal CVH (11) that focused on health behavior and health components of CVH in the absence of clinically manifested CVD. The goal was to improve CVH of all Americans by 20% and reduce mortality from cardiovascular diseases and stroke by 20% until 2020 (11).

The aim of the present review is to explain the concept of ideal CVH, and to present current research about the prevalence of ideal CVH, its sociodemographic inequalities and the association of ideal CVH with the incidence and mortality from CVD.

THE CONCEPT OF IDEAL CARDIOVASCULAR HEALTH

The AHA's concept of ideal CVH is focused on two groups of factors (11). The first group consists of four behavioral components of CVH (smoking, body mass index, physical activity and diet), and in the second group there are four health (biological) components of CVH (smoking, total cholesterol, blood glucose and blood pressure). Given the importance of smoking abstinence and smoking cessation for improving health, smoking is included in both lists, the list of health behavior factors and the list of health factors (11). The criteria for classifying CVH components into categories of ideal, average and poor CVH were determined and presented in **Table 1**.

Ideal CVH is defined as the simultaneous presence of seven ideal CVH components or "Life's Simple 7" (non-smoking, body mass index <25 kg/m2, level of physical activity up to the achievement of the set goal, diet in accordance with national recommendations, blood pressure <120/80 mm Hg, total cholesterol <200 mg/dL and blood glucose level <100 mg/dL) in the absence of clini-

Table 1. AHA definitions of poor, average, and ideal CVH components in adults (≥20 years) (11).

7 CVH components (Life's simple 7)	CVH categories		
	Poor	Average	Ideal
Smoking	Current	Former, quit ≤12 months	Never or quit >12 months
Body mass index	≥30 kg/m2	25-29.99 kg/m2	<25 kg/m2
Physical activity	No exercise	1-149 min of moderate exercise or 1-74 min of vigorous exercise/week	150+ min of moderate exercise or 75+ min of vigorous exercise/week
Diet (score components*)	0-1 components of a healthy diet	2-3 components of a healthy diet	4-5 components of a healthy diet
Total cholesterol	≥240 mg/dL	200–239 mg/dL or treated to goal	<200 mg/dL, untreated
Blood pressure	SBP ≥140 or DBP ≥90 mmHg	SBP 120–139 mmHg or DBP 80–89 mmHg or treated to goal	SBP/DBP <120/80 mmHg, untreated
Fasting glucose	≥126 mg/dL	100–125 mg/dL or treated to goal	<100 mg/dL, untreated

CVH: cardiovascular health
SBP: systolic blood pressure; DBP: diastolic blood pressure.
*Include: fruits and vegetables ≥4–5 servings/day; fish ≥2 servings/week; whole grains ≥3 servings/day; sodium ≤1500 mg/day; sweetened soft drinks ≤450 kcal/week.

cally manifested CVD (including IHD, stroke, heart failure, etc.) (11). Average

CVH is defined as the presence of at least one component of CVH at an average level, with no poor components, and poor CVH as the presence of at least one poor component (12).

A certain number of authors (13-15) used the total CVH score to assess CVH. Each ideal CVH component was assigned 2 points, the average component was assigned 1 point, and the poor component was assigned no points (0). The sum of the points of all 7 components of CVH gives the total score of CVH with a range from 0 (all components categorized as poor) to 14 points (all components at an ideal level). Depending on the size of the score, CVH was evaluated as ideal (10-14 points), average (5-9 points) and poor (0-4 points).

In the systematic review of the literature on the prevalence of CVH, Younus et al. (16), based on the available data of analyzed studies, assessed CVH as ideal (6–7 ideal components), average (2–5 ideal components) and poor (0–1 ideal component).

When comparing the results of different studies, it is important to keep in mind these different criteria in evaluating ideal, average, and poor CVH.

In early 2022, AHA introduced an enhanced CVH assessment tool named "Life's Essential 8" (17). It included all components of "Life's Simple 7" (revised diet, nicotine exposure, blood glucose, and lipids), with the addition of sleep health. The foundational context of mentioned approach is the advanced social-ecological model that integrates a variety of structural and socioeconomic determinants of health, providing the framework that investigates the ability to optimize CVH on individual and community levels. Sleep has a multidimensional association with all seven CVH metrics, indirectly affecting CVH and there is an evidence on sleep hygiene effects on coronary heart disease (CHD) with poor sleep hygiene having adverse effects on CHD (17-19).

PREVALENCE OF CARDIOVASCULAR HEALTH CATEGORIES

The prevalence of ideal adult CVH (all 7 components with ideal values) is low worldwide and is less than 1% in the USA (12), China (20) and Spain (21), and the lowest values (0.02% and 0.1%) were recorded in the Republic of Srpska, Bosnia and Herzgovina (22-23) and Serbia (24), respectively.

If the assessment of the prevalence of ideal CVH is based on a milder criterion (a simultaneous presence of six and seven components at the ideal level), the values are higher. A systematic review and meta-analysis by Jankovic et al. (25) showed a low prevalence of ideal CVH in all analyzed countries (ranged from 0.5% in the USA to 15% in China). The majority of studies reported ideal CVH prevalence below 5% (like 4.1% in Serbia). The prevalence of average CVH ranged from 70% in the USA to 93% in Serbia, while the lowest and highest prevalence of poor CVH were 1% (in Korea) and 29% (in the USA), respectively. Another systematic literature review (16) that included fifty studies published between 2010 and 2015 reported a low prevalence of people with six and seven ideal components of CVH. The lowest prevalence of ideal CVH was found in Iran (0.3%), whereas the highest was found in China (15%). Also, numerous studies have been conducted in the USA, and the prevalence of ideal CVD ranged from 0.5% among African Americans to 12% among health care workers in South Florida (16). Differences in CVH categories' prevalence in several studies based on a milder criterion are presented in **Figure 1**.

The prevalence of participants who achieved all four ideal health behaviors was lower compared to those with all four biological metrics (25). For example, in a Serbian study (24) only 0.4% of the population had all four ideal behavioral factors compared to 9.1% of the population with all four ideal biological factors, and this pattern was more prevalent among women than among men in both CVH component groups.

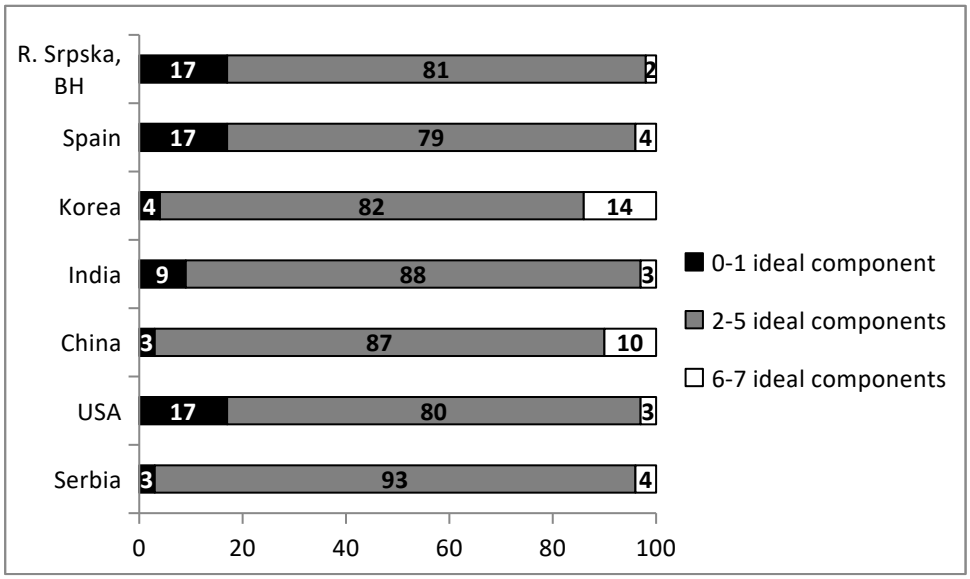

Figure 1. Prevalence (%) of ideal, average and poor CVH* in different countries

*modified by Younus et al., 2016.
poor CVH: 0-1; average CVH: 2-5; ideal CVH: 6-7 ideal CVH component

Regarding all seven ideal CVH metrics independently, a healthy diet was in most cases the least represented component, despite different assessment criteria, and almost all studies showed that more than 50% of the population had an ideal smoking status (i.e., no smoking for at least one year), which is most likely the result of successful smoking prevention at the global level (16). The results by Jankovic et al. (24) showed that the least prevalent ideal CVH component was an ideal diet (2.4%), and the most prevalent ideal glucose, i.e., absence of diabetes (92.2%). 86.4% of respondents had ideal cholesterol (absence of elevated cholesterol values), 63.8% non-smoking status, 52.7% ideal physical activity, 40.4% ideal BMI and ideal blood pressure was found in 17.5% of individuals. Similarly, Stojisavljevic et al. (26) found that poor diet (59.6%) was the most prevalent CVH component followed by poor physical activity (40.0%), while the least prevalent was poor fasting blood glucose (6.9%).

SOCIODEMOGRAPHIC INEQUALITIES IN CARDIOVASCULAR HEALTH

Findings from numerous studies conducted in developed and developing countries (middle- and low-income) and assessing CVH in an adult population showed that women had better ideal CVH compared to men, greater number of ideal CVH components, ideal behavioral and health factors (22, 27-29). Jankovic et al. (24) demonstrated that women had almost four times higher ideal CVH (simultaneous presence of 6 and 7 components at an ideal level) prevalence than men (6.2%:1.6%). Compared to men, women also had a higher prevalence of ideal smoking status, ideal diet, ideal BMI, and ideal blood pressure, and a lower prevalence of ideal physical activity, ideal cholesterol, and ideal glucose (21,23-24).

Over the past decade and a half, significant progress has been made in the field of cardiovascular risk factors in people of both sexes. Although the classic risk factors are the same for men and women, the findings of many studies indicate that their impact in women cannot be equated with that in men (30-31). Consequently, CVD prevention strategies should be different for individuals of different sexes.

The concept of CVH can be used in the future as a tool for easier understanding of gender differences in cardiovascular risk factors. Findings about gender inequalities in the prevalence and number of ideal CVH components should be used to develop appropriate CVH prevention policies adapted to the needs of both sexes.

Regarding age inequalities in ideal CVH, the highest prevalence of the ideal CVH (6 and 7 ideal components) was observed among the youngest (20-22,28-29,32). Also, in a Serbian study (24) the youngest age group (20-39 years) had the ideal CVH most frequently (9.0%), which is almost four times higher in people from the middle age group (2.3%) and nine times higher (1.0%) in people over 65 years old.

A similar gradient was observed in the values of ideal health index (all four ideal health factors) of CVH (18.8%: 5.6% :2.7%). When it comes to ideal behavioral index (all four ideal health behaviors), it was the best among the oldest participants (0.7%) compared to the youngest (0.4%) and middle-aged participants (0.3%) (24). The percentage of subjects who had four, five, and six ideal components of CVH decreased with age and was the lowest in the oldest age group (20-22, 28-29).

There is scarce literature on the inequalities in CVH between urban and rural areas. Del Brutto et al. (33) reported better CVH of the rural population of Ecuador than the CVH of the urban population of the USA and concluded that these differences were associated with

a healthier lifestyle in a rural environment. Despite the better behavioral index of the population of rural areas of the Republic of Srpska, Janković et al. (22) found that CVH was better among the residents of urban areas. The results of another study (24) showed that there was no significant difference in ideal CVH regarding the type of settlement.

Ideal CVH was more common in individuals living without a partner (single, divorced, widows) compared to individuals living with a partner (married or cohabiting). In literature, living without a partner is inconsistently associated with cardiovascular risk, although there is significant epidemiological evidence that social isolation can affect emotional stress mediated by neurohormones, health behaviors, and access to health care, resulting in an association with cardiovascular risk (34-37). In a large international cohort of middle-aged outpatients, living without a partner was independently associated with an increased risk of total and CVD mortality (38). In a study in the Republic of Srpska, living without a partner was associated with a greater number of ideal CVH components and ideal biological components, but not with ideal behavioral components (22). On the contrary, in China (39) those who were married or lived with a partner had two times better CVH compared to those living without a partner.

Concerning socioeconomic inequalities in CVH numerous studies (21,24,39-40) showed that ideal CVH was positively related to education. Respondents with high education had better CVH (ideal CVH, ideal behavioral index and ideal health index) than those with lower educational attainment. In two cross-sectional studies conducted in the USA (32,41), ideal CVH was highest in the most educated individuals. Graziani et al. (21) showed in Spain that the least educated people had the worst CVH and compared to them only the oldest Spaniards had worse CVH. Data from six cross-sectional studies conducted in Denmark from 1978 to 2006 showed a greater upward trend in ideal CVD among persons with a high educational level (42). However, the Danish authors did not use the AHA criteria to define CVH. In a Serbian study (24) people with higher education were more prone to higher prevalence of ideal BMI, ideal diet, and ideal blood pressure. The mentioned educational inequalities in CVH can be attributed to the fact that education provides access to important health-related resources (43), which makes more educated individuals more skilled in dealing with everyday issues relevant to their health (within the family, social and work environment).

Positive gradient was also observed between socioeconomic status measured by the wealth index and ideal CVH (24). The prevalence of ideal CVH, ideal health index, ideal healthy diet, and ideal blood pressure increased in rich residents compared to poor residents. However, no relationship was found between CVH and the wealth index in the Republic of Srpska, Bosnia and Herzegovina (15). In a study conducted in China, higher socio-economic status was independently associated with a higher prevalence of five or more ideal CVH components but only in women (44). A possible explanation is that the most vulnerable groups of residents have insufficient material and social resources needed to improve living conditions. Another explanation could be the epidemiological transition from "diseases of the rich" to "diseases of the poor" (45).

ASSOCIATION OF CARDIOVASCULAR HEALTH WITH THE INCIDENCE AND MORTALITY FROM CARDIOVASCULAR DISEASES

The results of a meta-analysis of nine cohort studies that included 12.878 subjects (46), as well as a systematic review of the literature (16), showed that ideal CVH was inversely related to the incidence and mortality rates of CVD, as well as overall mortality rates. People with the presence of a greater number of ideal CVH metrics, (5, 6, and 7 ideal components of CVH) are significantly less likely to suffer from CVD, IHD and stroke and have lower mortality from CVD, as well as total mortality compared to people with no or only one ideal component of CVH (12, 47-51). Ideal CVH is a predictor of a lower risk of myocardial infarction (MI), stroke and fatal cardiovascular outcomes among whites, blacks, and Hispanics (52).

Recent data have demonstrated a significantly lower risk when comparing ideal to poor CVH (51,53). Radovanovic et al. (53) showed in their systematic review and meta-analysis of prospective studies that there was a lower risk of developing composite CVD, CHD, MI, and stroke of 76%, 78%, 82%, and 62%, respectively. Similarly, Ramírez-Vélez et al. (51) reported a lower risk for developing composite CVD, CHD, MI, and stroke of 77%, 79%, 76%, and 67%, respectively.

The status of the CVH in the population is essential for predicting the risk of getting sick and dying from CVD, which can be reduced by improving both health, i.e. biological factors of CVH (blood pressure, total cholesterol, blood glucose), and behavioral factors of CVH (smoking, diet, obesity and physical activity).

CONCLUSION

Assessment of CVH is a relatively new approach that encourages the population to achieve the set goals (normal blood pressure, normal blood cholesterol level, the absence of diabetes, normal weight) and to adopt a healthy lifestyle (non-smoking, adequate physical activity, healthy diet), which contributes to the CVH improvement. This approach emphasizes the importance of preventing risk factors responsible for the development of CVD.

In order to help individuals and populations to improve their CVH, it is necessary to create comprehensive and specifically tailored strategies and interventions at the individual and population level. It is possible to improve the CVH of the population by reducing the prevalence of poor and increasing the prevalence of ideal behavioral and health components of CVH, which can be achieved with a healthy lifestyle and adequate therapy. The methods and techniques used in health education in order to improve people's lifestyle and adherence to prescribed therapy have proven to be not only effective, but also economically profitable.

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KONCEPT I ZNAČAJ IDEALNOG KARDIOVASKULARNOG ZDRAVLJA

Janković Janko[1]

Sažetak

Kardiovaskularne bolesti (KVB) su vodeći uzrok smrti širom sveta. U 2019. godini, 17,9 miliona ljudi je umrlo od KVB, što čini 32% svih smrtnih slučajeva globalno. Opterećenje KVB u datoj populaciji je povezano sa kardiovaskularnim zdravljem (KVZ) te populacije. Američka asocijacija za srce je 2010. godine osmislila novi koncept idealnog KVZ koji se fokusira na dve grupe, bihejvioralne komponente (pušenje, indeks telesne mase, fizička aktivnost i ishrana) i zdravstvene komponente (ukupni holesterol, glukoza u krvi i krvni pritisak). Idealno KVZ je definisano kao istovremeno prisustvo sledećih sedam idealnih komponenti KVZ ili „Life's Simple 7" (nepušenje, indeks telesne mase <25 kg/m^2, nivo fizičke aktivnosti do ostvarenja postavljenog cilja, ishrana u skladu sa nacionalnim preporukama, krvni pritisak <120/80 mm Hg, ukupan holesterol <200 mg/dL i nivo glikoze u krvi <100 mg/dL) u odsustvu klinički ispoljene KVB. Prevalencija idealnog KVZ je niska u svetu i manja je od 1% u SAD, Kini i Španiji, a najniže vrednosti zabeležene su u Republici Srpskoj, BiH (0,02%) i Srbiji (0,1%). Idealno KVZ je obrnuto povezano sa stopama incidencije i mortaliteta od KVB, kao i sa stopama ukupnog mortaliteta. Osobe sa većim brojem idealnih komponenti KVZ (5, 6, i 7) značajno ređe boluju od ishemijske bolesti srca i šloga i imaju niži mortalitet od KVB, kao i ukupni mortalitet u poređenju sa osobama bez ijedne ili sa samo jednom idealnom komponentom KVZ. Status KVZ u populaciji je od suštinskog značaja za predviđanje rizika oboljevanja i umiranja od KVB, koji se može smanjiti poboljšanjem zdravstvenih/bioloških, kao i bihejvioralnih komponenti KVZ.

Ključne reči: idealno kardiovaskularno zdravlje, kardiovaskularne bolesti, komponente kardiovaskularnog zdravlja, Life's Simple 7, prevalencija, smrtnost

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ORIGINAL ARTICLE

Major adverse cardiac events in vascular surgery patients with peripheral arterial disease

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Summary

Introduction: Major adverse cardiac events (MACE) are the primary cause of morbidity and mortality in patients undergoing vascular surgical procedures. This is why it is essential to understand the relationship between coronary artery disease and peripheral arterial disease. In this paper, we aimed to determine predictors and risk factors related to the occurrence of MACE in patients undergoing vascular surgery for peripheral arterial disease.

Material and Methods: This is a retrospective study of 310 in-hospital patients treated at a high-volume vascular centre. The outcome was classified through the three-point MACE (myocardial infarction, stroke and cardiovascular mortality).

Results: We found that the majority of initial MACE happened in patients with aortobifemoral bypass surgery compared to other types of reconstruction (p=0.009). No significant correlation was established between the procedures performed and the short-term primary outcome. In the initial period, MACE was detected in 14 (5.8%) patients. Among them, six (1.9%) experienced a myocardial infarction (MI), three (1%) had a stroke, and five (1.6%) died within the first 30 days. All deaths were linked to cardiac ischemia, which ultimately culminated in cardiogenic shock and subsequent death. Short term MACE was observed in 2 (0.6%) patients both of whom had an MI.

Conclusion: Major vascular surgery in patients with coronary artery disease is a highly morbid procedure and perioperative MACE places them at a significantly high risk of mortality. Early detection of coronary heart disease and preoperative optimization can play a major role in reducing the risk of MACE.

Keywords: peripheral arterial disease, coronary artery disease, MACE, myocardial infarction, stroke

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INTRODUCTION

Atherosclerosis is a progressive and diffuse inflammatory disease characterized by the accumulation of lipids and fibrous elements in the arteries (1). The accumulation of these metabolic products and a subsequent inflammatory response leads to the narrowing of the arterial blood vessels and reduced oxygen supply to the tissues (2). This can lead to different clinical manifestations depending on the involved vascular beds (3–5). Both coronary artery disease (CAD) and peripheral arterial disease (PAD) represent a manifestation of atherosclerosis with coronary complications being the primary cause of postoperative morbidity and mortality in patients undergoing surgical treatment for PAD (6,7). Although it is commonly assumed that patients with severe PAD have concurrent and severe CAD as well, the prevalence of significant CAD in patients with severe PAD varies widely, with figures ranging between 30% and 90% (7).

Every other inhabitant of the Republic of Serbia dies from cardiovascular disease. Compared to 2020, when mortality from heart and blood vessel diseases was 801.6 per 100,000 inhabitants, in 2021 the mortality rate increased to 828.3 per 100,000 inhabitants (8). With an ever-ageing population, we can expect a further increase in morbidity and mortality of cardiovascular diseases in the following decades.

Major adverse cardiac events (MACE) are used as composite outcomes in randomized controlled trials and observational studies (9). According to the United States Food and Drug Administration and European Medicines Agency, the three-point MACE outcome consists of acute myocardial infarction (AMI), stroke, and total cardiovascular mortality (10). Furthermore, four-point MACE and five-point MACE outcomes are used as well, adding hospitalization for unstable angina or revascularization procedures and heart failure (9,11).

It is essential to understand the relationship between CAD and PAD since MACE are the primary cause of morbidity and mortality in patients undergoing vascular surgical procedures(12,13).

In this paper, we aimed to determine predictors and risk factors related to the occurrence of MACE in patients undergoing vascular surgery for PAD.

METHODS

This is a retrospective study of 310 in-hospital patients treated at a high-volume vascular centre from January 2022 to June 2022. We obtained data from available medical records. All patients provided informed consent, and the Ethical Committee of the Institution approved the study. The data included 1) basic demographic data, 2) clinical presentation and course of the disease, 3) preoperative and postoperative therapy, 4) type of vascular procedure, 5) coronary angiography findings, 6) echocardiographic findings, and 7) primary outcome classified through the three-point MACE (AMI, stroke and cardiovascular mortality). The patients were followed up for 6 months. We analyzed the primary outcome at 30 days (initial) and 6 months (short-term) (14,15).

Risk factor-related data included 1) previous myocardial infarction (MI) within the past 5 years, 2) angina pectoris (AP), 3) previous myocardial revascularization procedures (percutaneous coronary intervention – PCI and coronary artery bypass grafting – CABG), 4) hypertension (HT), 5) hyperlipoproteinemia (HLP), 6) diabetes mellitus (DM), 7) chronic obstructive pulmonary disease (COPD), and 8) chronic kidney disease (CKD) and creatinine levels. We used The Vascular Study Group of New England Cardiac Risk Index (VSG-CRI) and the Revised Cardiac Risk Index (RCRI) based on the criteria presented in **Table 1** (16–18).

Table 1. Risk indexes and the scoring system used in the study

RCRI		VSG-CRI	
Factor	**Score**	**Factor**	**Score**
Elevated-risk surgery (intraperitoneal, intrathoracic, suprainguinal, vascular)	1	**Age**	
		≥80	4
		70-79	3
		60-69	2
History of IHD (History of MI, history of positive exercise test, current chest pain considered due to myocardial ischemia, use of nitrate therapy or ECG with pathological Q waves)	1	**History of IHD**	2
History of CHF (Pulmonary oedema, bilateral rales or S3 gallop, paroxysmal nocturnal dyspnoea, CXR showing pulmonary vascular redistribution)	1	**History of CHF**	2
History of cerebrovascular disease (prior TIA or stroke)	1	**COPD**	2
Preoperative treatment with insulin	1	**Insulin dependent diabetes**	1
Preoperative creatinine >176.8 μmol/L	1	**Preoperative creatinine >160 μmol/L**	2
		Smoking	1
		Long term β-blocked	1
		History of CABG and PCI	-1

RCRI: Revised Cardiac Risk Index, VSG-CRI: Vascular Study Group of New England Cardiac Risk Index, IHD: Ischemic Heart Disease, MI: Myocardial infarction, ECG: electrocardiogram, CHF: congestive heart failure, CXR: Chest X-ray, TIA: Transient ischemic attack, COPD: chronic obstructive pulmonary disease, CABG: coronary artery bypass grafting, PCI: percutaneous coronary intervention.

Statistical analysis

We analyzed data by parametric and nonparametric methods. Normality was tested using Kolmogorov–Smirnov test and Q-Q diagrams. The observed characteristics were expressed as mean values, standard deviation, median, and interquartile range (IQR). We used the Mann–Whitney U test for continuous nonparametric data, whereas Student's t-test was used for continuous parametric data. Categorical data were analyzed using the Chi-square test and Fisher exact test, to determine the statistically significant difference. Significance was set at a 2-sided $p<0.05$. IBM SPSS Statistics 26 (Armonk, New York, USA) was used for the analysis.

RESULTS

Demographic parameters and risk factors are given in **Table 2**. No significant difference was observed regarding the frequency of risk factors and sex ($p>0.05$). A significant proportion of patients (>25%) had undergone a prior myocardial revascularization procedure before the admission, and almost 15% had experienced a prior MI. Diabetes mellitus was highly prevalent, affecting >50% of patients, while 58 (32.7%) diabetic patients were insulin dependent. The majority of patients had complaints on admission that according to Fontaine could be classified as category II (intermittent claudication), rest pain (Fontain III) was reported by 62 (20%) patients, while the presence of wound and tissue loss (Fontain IV) was reported in 91 (29.4%) patients. Significantly more patients with DM had complaints in Fontaine IV category compared to patients who did not suffer from DM ($p=0.001$).

A significant majority of patients, exceeding 75%, underwent aortobifemoral bypass surgery. Iliacofemoral reconstruction was undertaken in 2.3% of patients, while above-the-knee femoropopliteal (FP) bypass was performed in 9.7%, and below-the-knee FP bypass in 6.5%. We found that the majority of initial MACE happened in patients with aortobifemoral bypass surgery compared to other types of reconstruction ($p=0.009$). No significant correlation was established between the procedures performed and the short-term primary outcome. However, it was noted that patients who received below-the-knee FP bypass required additional interventions like necrotomy and toe amputation in comparison to patients who underwent other types of reconstructions.

Table 2. Demographic and risk factors

Factor	N (%)
Age (years) *(median, min-max)*	66 (38-86)
BMI (kg/m²) *(median, min-max)*	25.79 (17-42.6)
Male sex	221 (71.3)
Previous MI	45 (14.5)
Angina pectoris	29 (9.4)
CHF	64 (20.7)
Fontain grade	
II	157 (50.6)
III	62 (20)
IV	91 (29.4)
HLP	267 (86.1)
HTA	282 (91)
DM	177 (57.1)
Previous myocardial revascularization	
PCI	43 (13.9)
CABG	36 (11.6)
History of smoking	249 (80.3)
CKD	12 (3.9)
COPD	20 (6.5)
Previous TIA/CVI	30 (9.7)
RCRI *(median, min-max)*	1 (1-5)
VSG-CRI *(median, min-max)*	4 (2-12)

RCRI: Revised Cardiac Risk Index, VSG-CRI: Vascular Study Group of New England Cardiac Risk Index, IHD: Ischemic Heart Disease, MI: Myocardial infarction, ECG: electrocardiogram, CHF: congestive heart failure, CXR: Chest X-ray, TIA: Transient ischemic attack, COPD: chronic obstructive pulmonary disease, CABG: coronary artery bypass grafting, PCI: percutaneous coronary intervention.

Table 3. Performed open surgical procedures

Surgical procedure	N (%)
Aortobifemoral bypass surgery	234 (75.5)
Iliacofemoral reconstruction	7 (2.3)
Above-the-knee femoropopliteal bypass	30 (9.7)
Below-the-knee femoropopliteal bypass	20 (6.5)
Other	19 (6.1)

In the initial period, MACE was detected in 14 (5.8%) patients. Among them, six (1.9%) experienced a myocardial infarction (MI), three (1%) had a stroke, and five (1.6%) died within the first 30 days. All deaths were linked to cardiac ischemia, which ultimately culminated in cardiogenic shock and subsequent death. Short term MACE was observed in 2 (0.6%) patients both of whom had an MI. Significant risk factors related to initial MACE are given in **Table 4**. No factors were found to significantly contribute to short-term MACE in our cohort.

Table 4. Risk factors for the occurrence of initial major adverse cardiac event

Factor	OR	95% CI	p
Age >65 years	1.075	1.038-1.113	<0.001
Creatinine level	1.005	1.001-1.009	0.015
CKD	4.063	1-976-8.354	<0.001
Dialysis	3.635	1.149-11.504	0.028
Previous myocardial revascularization	2.789	1.2-6.481	0.017
Emergency admission	4.968	2.297-10.748	<0.001

CKD: chronic kidney disease

The median calculated RCRI was 1 and VSG-CRI was 4 for the entire cohort. There was a significantly higher RCRI score in patients who had initial MACE (3 vs. 1; $p<0.001$). Same was observed for VSG-CRI score (5 vs. 2; $p=0.011$).

DISCUSSION

A study by Thomas et al. reported MACE in patients undergoing major vascular surgery to be as high as 11% with overall mortality of 6.4% (56.2% mortality in all patients with MACE) (19). However, their cohort consisted of patients with known CAD making this a high-risk group of patients. We already analyzed the prevalence of CAD in vascular surgery patients and the implications of preoperative coronary angiographies and myocardial revascularization and found significant prevalence of CAD in PAD patients (20). Furthermore, Thomas et al. found a significant difference in the occurrence of MACE in patients with prior revascularization compared to nonrevascularized patients (22% vs. 7%). Other studies, however, have found that the presence of a coronary stent (i.e., prior myocardial revascularization) is a predictor of MACE and that these patients should be carefully managed. We obtained similar results in our cohort, where it was shown that prior myocardial revascularization was a significant predictor of an adverse cardiac event. This can be explained by hemodynamic changes during operations on the aorta and large blood vessels. A detailed subcohort analysis is needed to better stratify these patients.

In the study by Smeili et al., the risk of MACE was related to advanced age, obesity, reduced functional lung capacity, renal impairment, aortic surgery, and altered troponin levels. RCRI and VSG-CRI had an area under the curve of 0.635 and 0.639, respectively, for early cardiovascular complications and 0.562 and 0.610 for 30-day death (21). The reported MACE in this study is lower compared to the available data in literature (19). Overall mortality of 1.6% can be considered an excellent result, but a more detailed analysis of subgroups of patients is necessary for a better stratification of risk factors and better management in the perioperative period.

The association of MACE with vascular surgical treatment can be explained in several ways. This may be due to large intra- and extravascular fluid shifts, prolonged duration of aortic cross-clamping during open surgical treatment of aortic disease, large volume loss and insensible fluid losses, as well as the induction of hypothermia (22). This is associated with an enhanced stress response, characterized by elevated levels of circulating cortisol and catecholamines in the perioperative period, promoting a hypercoagulable state and an increased myocardial oxygen demand (23). In addition to the risks inherent in the surgery itself, patients are at high risk of developing MACE due to the underlying medical comorbidities prevalent in this patient population, all of which are associated with atherosclerosis (24).

Limitations

Our study has some limitations. Primarily, due to the intrinsic characteristics of observational studies, the capacity to establish causal relationships remains limited. In addition, the analysis relies exclusively on a group of individuals admitted to hospital, thus mitigating its generalizability to a wider spectrum of PAD patients. Our decision to include consecutive patients was based on their representation in the wider population of vascular patients encountered in our clinical practice.

CONCLUSION

Major vascular surgery in patients with coronary artery disease places them in high-risk group of mortality due to the occurrence of perioperative MACE. Factors such as age > 65 years, elevated creatinine levels, chronic kidney disease and emergency admission played a significant role in the development of perioperative MACE. Early detection of coronary heart disease and preoperative optimization can play a major role in reducing the risk of MACE.

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VELIKI NEŽELJENI KARDIOVASKULARNI DOGAĐAJI KOD VASKULARNIH HIRURŠKIH PACIJENATA SA PERIFERNOM ARTERIJSKOM BOLEŠĆU

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Sažetak

Uvod: Veliki neželjeni srčani događaji (*MACE*) su primarni uzrok morbiditeta i mortaliteta kod pacijenata koji su podvrgnuti vaskularnim hirurškim procedurama. Zbog toga je neophodno razumeti vezu između koronarne bolesti i periferne arterijske bolesti. Cilj ovog rada je utvrđivanje prediktora i faktora rizika koji se odnose na pojavu velikih neželjenih srčanih događaja kod pacijenata koji su podvrgnuti vaskularnoj hirurgiji zbog periferne arterijske bolesti.

Materijal i metode: Ovo je retrospektivna studija na 310 bolničkih pacijenata lečenih u vaskularnom centru velikog obima. Ishod je klasifikovan kroz tri tačke (infarkt miokarda, moždani udar i kardiovaskularni mortalitet).

Rezultati: Otkrili smo da se većina *MACE* desila kod pacijenata sa aortobifemoralnim bajpasom u poređenju sa drugim tipovima rekonstrukcija (p=0,009). Nije ustanovljena značajna korelacija između urađenih zahvata i kratkoročnog primarnog ishoda. U početnom periodu *MACE* je otkriven kod 14 (5,8%) pacijenata. Među njima, šest (1,9%) je doživelo infarkt miokarda (IM), troje (1%) je imalo moždani udar, a pet (1,6%) je umrlo u prvih 30 dana. Svi smrtni slučajevi su bili povezani sa srčanom ishemijom, koja je na kraju kulminirala kardiogenim šokom i kasnijom smrću. Kratkotrajni *MACE* je primećen kod 2 (0,6%) pacijenta od kojih su oba imala IM.

Zaključak: Velika vaskularna hirurgija kod pacijenata sa koronarnom bolešću je visokorizična procedura i perioperativni *MACE* ih stavlja u značajno visok rizik od smrtnosti. Rano otkrivanje koronarne bolesti srca i preoperativna optimizacija mogu igrati glavnu ulogu u smanjenju rizika od *MACE*.

Ključne reči: periferna arterijska bolest, koronarna bolest, MACE, infarkt miokarda, moždani udar

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CASE REPORT

Extrarenal Wilms tumor mimicking funicular hydrocele

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Summary

Introduction/Objective: Extrarenal Wilms' tumor (ERWT) comprises 0.5% to 1% of all nephroblastoma cases. The most common locations of ERWT are the retroperitoneum, inguinal, lumbosacral and pelvic region, female genital organs, mediastinum and chest wall, spermatic cord and paratesticular region. ERWT most likely originates from the ectopic nephrogenic rest that undergoes a malignant transformation. The exclusion of primary renal tumor is necessary to establish the diagnosis of ERWT.

Patient Review: A 15-month-old male was operated on because of a left-sided inguinal mass that clinically resembled a funicular hydrocele. The surgical exploration of the inguinal canal revealed a solid tumor located in the area of the external inguinal ring, measuring around 2cm in diameter, with no ingvinal hernia present. Complete surgical resection of the mass was performed. The histological structure of the tumor corresponded to non-anaplastic nephroblastoma (Wilms tumor), mixed type. After abdominal CT scan primary renal tumor was excluded and the diagnosis of ERWT was made. Because of microscopic tumor rests on the resection margin, the patient was treated according to the high-risk SIOP Wilms tumor protocol. The patient made a full recovery. During the 10-year follow-up, the boy has remained disease-free.

Conclusion: ERWT is mostly diagnosed after the surgical removal of the specimen. The clinical resemblance of the ERWT located in the inguinal region to common benign inguinal conditions in children may lead to the omission of detailed preoperative work-up. Most children with ERWT in the inguinal region have an excellent prognosis, although most of them require adjuvant chemotherapy and/or radiotherapy after surgery.

Keywords: extrarenal Wilms tumor, inguinal region, funicular hydrocele

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INTRODUCTION

Extrarenal Wilms tumor (ERWT) is a rare entity with the occurrence rate of 0.5% to 1% in all nephroblastoma cases (1). It was first described by Moyson et al. in 1961 (2) and since then only case reports and small case series describing ERWT were published. There are about a hundred cases of ERWT presented in literature. The most common locations of ERWT are the retroperitoneum, inguinal area, lumbosacral and pelvic region, female genital organs (including the uterus, cervix, vagina, and ovaries), mediastinum and chest wall, and spermatic cord and paratesticular region (1,3–12).

The pathogenesis of ERWT remains controversial. The most accepted theory claims that ERWT originates from the ectopic nephrogenic rest (ENR) that undergoes a malignant transformation. The nephrogenic rests originate from metanephric blastema that persists after 36 weeks gestational age. Besides, the inguinal and scrotal locations of ENR suggest that the origin might also be mesonephric tissue (1,13,14). ENR can undergo spontaneous regression, become dormant, or progress towards hyperplastic proliferation. Fortunately, the malignant transformation happens only in the minority of ENR (15). ERWT may also develop as a part of teratoma. However, since teratoid ERWT is derived from primordial germ cells, most authors consider it a different entity from isolated ERWT (1,14).

The diagnosis of isolated ERWT relies on three criteria: histopathological finding of Wilms tumor in extrarenal location, exclusion of primary renal neoplasm, and no evidence of teratoma elements within the tumor (16,17).

There is no specific staging system for ERWT. Most authors use the National Wilms Tumor Study Group staging system modified for ERWT **(Table 1)** (14,16,18).

Table 1. Modified National Wilms Tumor Study (NWTS) staging system for ERWT (16)

Stage I	Tumor contained within capsule; complete excision Tumor capsular surface intact; no residual tumor apparent beyond the margins of resection
Stage II	Tumor extends beyond capsule but is completely excised Regional extension of tumor; tumor biopsied or local spillage of tumor, no residual tumor apparent at or beyond the margin of excision
Stage III	Residual tumor; lymph node involvement, peritoneal contamination by tumor spillage or peritoneal implants; tumor not completely removable because of local infiltration into vital structures
Stage IV	Distant metastases

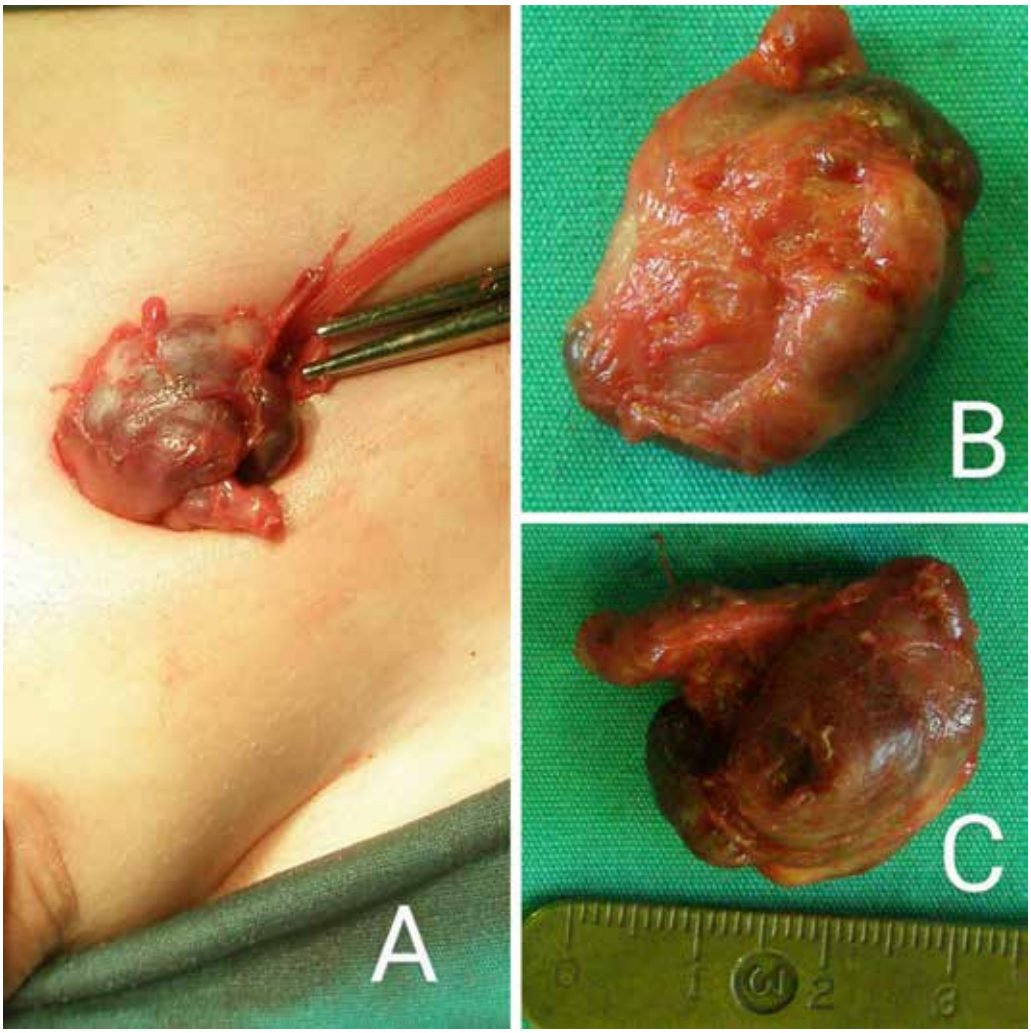

Figure 1. A. Intraoperative finding of the tumor mass located in the area of the external inguinal ring. Figure B, Figure C. Macroscopic appearance of the resected tumor.

CASE REPORT

A 15-month-old male was admitted to the Department of Abdominal Surgery for the operative treatment of the left inguinal mass which clinically resembled a funicular hydrocele. The mass was noticed by the boy's parents a month before admission. Otherwise, his general past medical history was unremarkable. The non-reducible and non-tender mass was located in the left inguinal region, with approximate dimensions of 2x3 cm. The physical examination on admission showed no other abnormalities. As the clinical findings strongly pointed to funicular hydrocele, no further diagnostics were performed. The surgical exploration of the left inguinal canal revealed a tumor located in the area of the external inguinal ring **(Figure 1A)**. No inguinal hernia or funicular hydrocele were present. A macroscopic complete resection of the mass with the preservation of the spermatic cord was performed. The child recovered uneventfully and was discharged on the second postoperative day.

The tumor weighed 5.2 grams and had dimensions of 2.6x2.2x1.5 cm, with a slightly lobulated surface **(Figure 1B and Figure 1C)**. On cross-section, the tumor tissue had a multinodular structure, brown-grey color, and soft-elastic consistency. According to the protocol of the International Society of Pediatric Oncology "Nephroblastoma Clinical Trial and Study - SIOP WT 2001" for tumors resected without preoperative chemotherapy, the histological structure of the tumor fully corresponded to Wilms' tumor, non-anaplastic, mixed type, which is classified into a group of childhood kidney tumors of intermediate prognostic risk. The borders of the tumor growth were very sharp, without signs of infiltrative growth, although no capsule was formed **(Figure 2A)**. There was a characteristic triphasic histological feature of the tumor with nodules of dense, small, blastemic cells with rare, focal differentiation into an epithelial component in the form of immature tubular structures. Among these structures there was a highly collagenized, stromal component of the tumor with individual, better differentiated, tubular, epithelial structures **(Figure 2B)**. There was no anaplasia of tumor cells nor necrosis of tumor tissue. However, tumor elements were found in dilated venules on the periphery of the section **(Figure 2C)**. Outside the tumor nodule, near the specimen resection margin, tiny lobules of mature fatty tissue and several small islands of immature glomeruloid structure, corresponding to ectopic nephrogenic remnants were found **(Figure 2D)**. There were no elements of teratoma within the tumor.

The primary renal tumor was excluded by an abdominal CT scan. Chest x-ray was free of lung metastases. In addition, a CT scan of the left inguinal region showed heterodense formation which could be attributed to post operative hypervascularity, but the local residual disease could not be excluded.

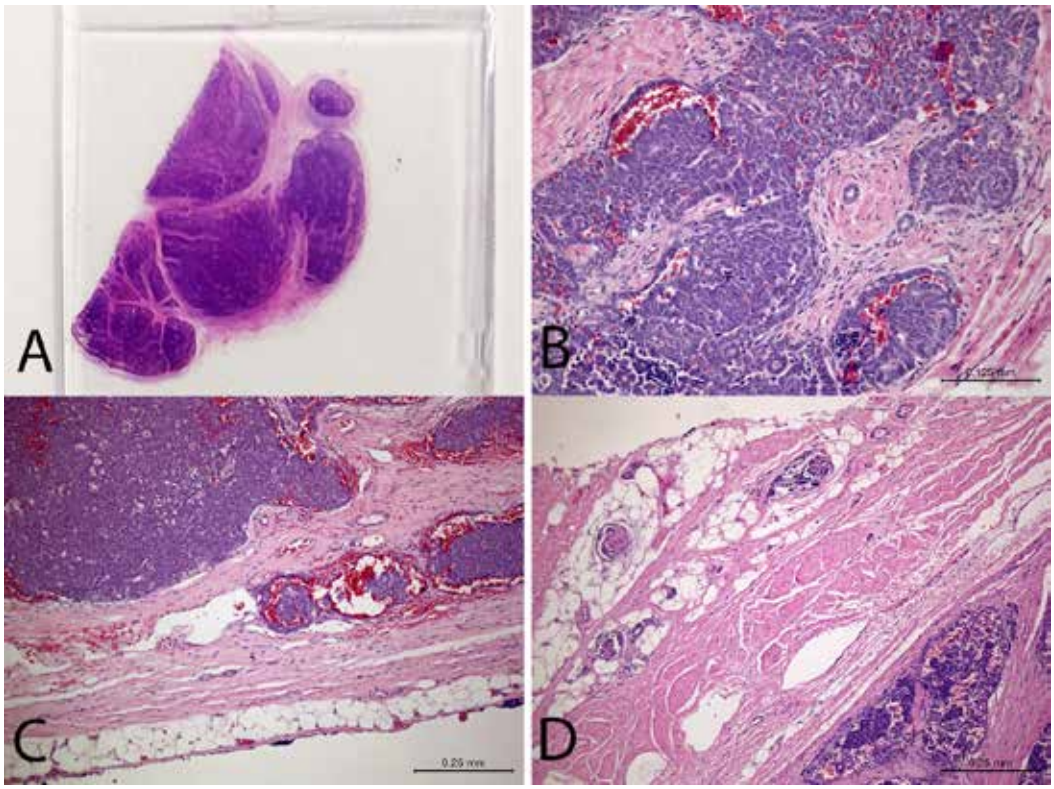

Figure 2. Histopathology of the tumor. **A.** Circumscript, non-encapsulated, multinodular pattern of tumor growth in a tissue section photographed on the microscopic glass slide. The width of the coverslip is 24 mm. **B.** Typical histological structure of Wilms tumor with a predominant, dense, small-cell blastema component; focal, tubular, epithelial differentiation; and a highly hyalinized, stromal component. (HE, x200) **C.** Vascular tumor invasion in dilated venules on the periphery of the tumor node. (HE, x100) **D.** In the upper left part of the image, in the mature, fatty tissue near the resection surface and outside the tumour nodule, three tiny islands whose glomeruloid histological structure corresponds to ectopic nephrogenic remnants can be observed. It is assumed that these could be precursors of extrarenal Wilms tumor. (HE, x100)

The patient was treated according to the SIOP 2001 Protocol for high-risk group of Wilms tumor. On final assessment, the patient was disease-free. During the 10 year follow-up no recurrence of the tumor was noted.

DISCUSSION

A literature search revealed 18 inguinal-located ERWT cases reported so far (19–34). Inguinal ERWT may mimic other more common benign diseases and malignant tumors located in this region. Our patient was misdiagnosed as having funicular hydrocele. Similarly, in three other reported cases patients were operated on for presumed inguinal hernia (29,34,35), and six tumors were found incidentally during routine orchidopexy (21,22,25,26,28,32).

In all analyzed cases, the diagnosis was obtained after surgical removal of the tumor. Like in our case, clinical resemblance to other pediatric inguinal conditions may lead to the omission of preoperative work-up. Among all reported cases, preoperative radiological assessment was done only in two: a CT scan was performed in a girl with inguinal swelling after herniectomy (24) and both a CT scan and F-18 FDG PET/CT were done in a 9-year-old boy with rapidly enlarging mass in the inguinal region (23). Even with a complete work-up, the rarity of this tumor and a lack of pathognomonic radiological features makes preoperative diagnosis virtually impossible (1,18).

Similarly to ERWT, ENR can be found in the inguinal region. There are 6 cases of the inguinal ENRs published so far. They are mostly encountered incidentally during hernia repair or orchidopexy (15). Most children with inguinal ENR had an uneventful postoperative follow-up with no recurrence (15,36). However, the patient presented by Cook et al. had local recurrence five months after ENR removal. In the specimen removed during the second operation, transformation into Wilms tumor was noted (22). Histological differentiation between ERWT and ENR can be difficult, especially if ENR is in the proliferative phase. Correct pathological diagnosis of ENR may help to avoid unnecessary adjuvant treatment. Nevertheless, close follow-up with clinical and ultrasound examinations of the inguinal region at least bi-annually is necessary (15,36).

The precise treatment protocols for ERWT remain elusive. Complete excision of the tumor is the primary goal of surgical treatment. The case presented by Luchtrach et al. demonstrated that nephrogenic rests and detached microscopic ERWT might be found in the seemingly unaffected soft tissue surrounding the tumor (29). In our case, based on a postoperative CT scan, it is possible that similar tumor rest, not visible during the operation, was present. Since the lesion regressed completely following chemotherapy, definitive histopathological confirmation of this claim is missing.

According to literature data, local recurrence of inguinal-located ERTW was present in three reported patients (19,35). They were all primarily treated with surgical excision alone. Time to recurrence ranged from 5 to 11 months. One of the patients, reported by Thomson et al. was treated with radiation therapy and made a full recovery. The other patient developed lung metastasis despite additional chemo- and radiotherapy and he died. The patient reported by Lail et al. was treated with re-excision and chemotherapy. The author reports complete remission, however, in a review by Coppes et al., it is stated that this patient developed brain metastases 24 months after surgery and was lost from follow-up afterwards (34).

All in all, there are four reports of children with distant metastases after inguinal ERWT - three had metastases in the lungs (19,23,30) and one in the brain (34). Tumor spread only to the local lymph node was reported by Groth et al. (26). We emphasize that metastases were either present initially, or they developed in children who had not been treated with adjuvant chemotherapy.

Fortunately, there are only two reported deaths after inguinal ERWT (19,30). Both reports with unfavorable outcome date from 40-50 years ago. On the contrary, in the case reported more recently by Jeong et al., lung metastases in a patient with ERWT were successfully treated with chemotherapy (23).

The aforementioned patient reported by Cooke et al. is the only child that was completely cured only with surgical removal of the tumor. In this case, a 0.5 cm focus of ERWT was found surrounded by ENR (22). Based on all presented data, adjuvant chemotherapy is advised in most patients with inguinal ERWT. It could be omitted only in exceptional cases with complete removal of ERWT with a portion of unaffected tissue around it.

ERWT prognosis is similar to the corresponding stage of renal Wilms tumor (16). It is proved that older age, larger tumor size, higher stage, unfavorable histology, and tumor spillage are associated with worse outcomes in ERWT (1,17). The inguinal location makes tumors detectable in the early stages of development, while still small in size. Additionally, ERWTs have predominantly favorable histology (1). Thus, the prognosis of most children with inguinal ERWT is excellent.

CONCLUSION

ERWT is a rare tumor, mostly diagnosed after surgical removal of the specimen. Clinical resemblance to common benign inguinal conditions in children may lead to the omission of more detailed preoperative work-up. The inguinal location makes tumors detectable while relatively small in size and most often without metastases. Most children with ERWT in the inguinal region have an excellent prognosis, although the majority of them require adjuvant chemotherapy and/or radiotherapy. Inguinal

ENR should be considered premalignant lesions and close follow-up after surgical removal is needed.

Author contributions

T.M. – the concept of the work, the acquisition, analysis and interpretation of data, preparing the draft of the manuscript
P.R. - the concept of the work, the acquisition, analysis and interpretation of data, preparing the draft of the manuscript
M.M. - the concept of the work, the acquisition, analysis and interpretation of data, interpretation of revised version of the manuscript
S.Đ. - the acquisition, analysis and interpretation of data, preparing the draft of the manuscript, interpretation of revised version of the manuscript
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D.Đ. - the acquisition, analysis and interpretation of data, interpretation of revised version of the manuscript
M.S. - the concept of the work, interpretation of revised version of the manuscript

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EKSTRARENALNI VILMSOV TUMOR KOJI JE IMPONOVAO KAO FUNIKULOCELA

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Sažetak

Uvod: Ekstrarenalni Vilmsov tumor (ERVT) predstavlja redak entitet koji čini 0,5% - 1% svih nefroblastoma. Ovaj tumor je najčešće lokalizovan u retroperitoneumu, ingvinalnoj, paratestikularnoj ili lumbosakralnoj regiji, ženskim genitalnim organima, kao i u medijastinumu ili zidu grudnog koša. Najveći broj autora smatra da ERVT nastaje malignom transformacijom ektopičnih primitivnih nefrogenih ostataka. U cilju postavljanja dijagnoze ERVT, neophodno je, pre svega, isključiti postojanje primarnog maligniteta bubrega.

Opis pacijenta: Dečak uzrasta 15 meseci operisan je zbog promene lokalizovane u levoj ingvinalnoj regiji koja je na osnovu kliničkog pregleda odgovarala funikuloceli. Intraoperativno funikulocela ili preponska kila nisu nađene, a u predelu spoljašnjeg igvinalnog prstena identifikovan je solidan tumorski čvor promera oko 2 cm i hirurški uklonjen u celini. Histopatološkim pregledom je ustanovljeno da tumor u potpunosti odgovara neanaplastičnom nefroblastomu (Vilmsovom tumoru), mešovitog tipa. Nakon CT pregleda abdomena isključeno je postojanje primarnog tumora bubrega i postavljena je dijagnoza ERVT. Zbog mikroskopskog ostatka tumorskih ćelija na resekcionoj margini, ordinirana je hemioterapija po protokolu za Vilmsov tumor visokog rizika. Po završetku lečenja, došlo je do potpune remisije koja se održala tokom desetogodišnjeg praćenja.

Zaključak: Dijagnoza ERVT se najčešće postavlja nakon hirurškog uklanjanja tumora. Zbog kliničke sličnosti ERVT lokalizovanih u ingvinumu sa mnogo češćim benignim patološkim promenama u ovoj regiji kod dece, detaljnije preoperativno ispitivanje najčešće izostaje. Najveći broj dece lečene zbog ERVT u ingvinalnoj regiji ima dobru prognozu, mada je kod većine pacijenata pored hirurške neophodna i adjuvantna hemio i/ili radioterapija.

Ključne reči: ekstrarenalni Vilmsov tumor, ingvinalna regija, funikulocela

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ORIGINAL ARTICLE

Development of random forest machine learning model for the detection of changes in liver tissue after exposure to iron oxide nanoparticles

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Summary

Introduction/Aim: The aim of our study was to create a machine learning model, specifically a random forest model, which uses textural data from liver micrographs to differentiate between normal hepatic tissue and damaged tissue exposed to iron oxide nanoparticles.

Material and Methods: Regions of interest in micrographs of hepatic tissue, obtained from mice treated with iron oxide nanoparticles and controls, were analyzed using the gray-level co-occurrence matrix (GLCM) method. The resulting GLCM features were employed as input data for the training and testing of the random forest model using the "Scikit-learn" library in the Python programming language. Additionally, a conventional decision tree model was developed, based on the classification and regression tree (CART) algorithm.

Results: The random forest model outperformed the alternative CART decision tree approach in terms of classification accuracy, correctly predicting the class for 73.67% of the instances in the validation ROI dataset. The area under the receiver operating characteristic curve was 0.81, indicating relatively good discriminatory power. The F1 score for the model was 0.74, showcasing fairly good precision and recall, though not perfect.

Conclusion: The data obtained from this study may be utilized for further development of artificial intelligence computation systems to identify physiological and pathophysiological changes in hepatic tissue. The results also serve as a starting point for additional research on the automation of histopathological analysis of liver tissue exposed to external toxic agents.

Keywords: artificial Intelligence, machine learning, iron oxide, liver

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INTRODUCTION

Rapid development of narrow artificial intelligence (AI) has led to a higher degree of automation in fundamental biomedical research as well as novel and innovative approaches to detection of physiological and pathophysiological phenomena. Nowadays, narrow artificial intelligence strategies based on supervised machine learning are often used for facilitation of signal analysis in various medical fields. Supervised machine learning is focused on presenting the machine (computer) with a series of examples with known input and output (target) data after which the machine learns to associate the data and identifies the complex patterns in data organization. Due to these new associations, subsequently developed machine learning algorithm acquires the ability to independently predict target data from new inputs. Some examples of supervised machine learning algorithms include the ones based on random forests, multilayer perceptrons, support vector machine and naive Bayes. All of these approaches can be used for versatile classification or regression tasks and have numerous potential applications in predicting analytics in medicine and biology (1-4).

Random forest algorithms are especially powerful and robust when used for classifying biological structures, based on experimental data derived from signal analysis. Random forest is an ensemble machine learning method that constructs a variety of decision trees connecting the input and target data. For classification tasks, trees in a Random Forest are typically constructed from bootstrapped samples of the original dataset and the final classification information is determined based on majority voting from these trees (5-9). This approach may provide high accuracy, especially when using data obtained from two-dimensional signals such as digital micrographs in pathology and pathophysiology. Also, such models can be both robust and flexible, they are resilient to overfitting data while at the same time they can handle a mix of categorical and numerical variables. In the past, random forest machine learning models were developed on numerous occasions to classify cells previously exposed to toxic substances and environments, and there are indications that they can be used as additional tools for differentiating normal from pathological structures in various experimental conditions (5, 6, 10).

Our previous research has shown that it is possible to develop a random forest model capable of separating normal from damaged cells based on gray-level co-occurrence matrix (GLCM) features (11). Gray-level co-occurrence matrix is essentially a contemporary and innovative signal analysis mathematical and second-order statistical technique used for extracting information from two-dimensional signals. Features such as angular second moment which is an indicator of textural uniformity and inverse difference moment as an indicator of local homogeneity can indeed serve as useful quantifiers of microscopic changes that cannot be observed even by most experienced microscopy experts. Previously, it has been shown that these features exhibit significant changes in liver tissue and cells exposed to potentially toxic chemical agents such as iron oxide nanoparticles (12). It has also been suggested that GLCM features could be used as inputs for random forest and other supervised machine learning algorithms to classify and predict discrete morphological and functional changes in the liver following the exposure to iron nanomaterials.

In this paper we have demonstrated that it is indeed possible to create a relatively accurate random forest model that uses GLCM data of liver micrographs for differentiation between normal and pathological tissue. We have shown that the model has relatively high discriminatory power in separating regions of interest of the liver tissue exposed to low dose of iron oxide nanoparticles from intact tissue despite their morphological similarity and a lack of conventional microscopic indicators of tissue damage. The model outperformed the alternative approach based on the classification and regression tree algorithm demonstrating its potential for further application in contemporary pathophysiology and pathology research.

MATERIAL AND METHODS

In this research we used the data obtained from 20 male, healthy C57BL/6 (C57 black 6, B6) mice divided into two groups (N=10). The experimental group received Iron (II,III) oxide nanoparticles (80-100 nm, Hongwu International Group Ltd. HWNANO materials, Guangzhou, Guangdong, CN) for 3 days, each day in a dose of 3 mg/kg. The controls received IP physiological solution for the same period. The study was a part of a PhD thesis research which received approval from both the Ethics commission for the protection and welfare of experimental animals of the University of Belgrade, Faculty of Medicine (approval number 229/2) and the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia - Veterinary Division (approval number 323-07-07783/2020-05). The procedures that followed were in line with the Universal Declaration of Animal Welfare (WSPA, London, 2000), the European Convention on the Protection of Vertebrates Used for Experimental and Other Scientific Reasons (1998), and various other domestic and international guidelines promoting responsible and compassionate treatment of experimental animals.

The liver tissue was acquired from all animals and fixated in Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) after which it was embedded in Paraplast, as previously described (13). The tissue sections were stained using the conventional Hematoxylin – Eosin technique. The digital micrographs

of liver tissue were obtained using Pro-MicroScan DEM 200 instrument (Oplenic Optronics, Hangzhou, CN) attached to OPTIC900TH Trinocular Biological Microscope (COLO LabExperts, Novo Mesto, Slovenia). The micrographs were sized 1200 x 1600 pixels with bit depth of 24 and horizontal and vertical resolution equaling 96 dpi **(Figure 1)**. Based on the micrographs, a total of 2000 Regions of interest (ROIs) were selected and analyzed: 1000 ROIs of the tissue exposed to IONPs and 1000 ROIs from controls. The ROI selection and GLCM analysis were done in Mazda software, previously created for COST B11 European project "Quantitative Analysis of Magnetic Resonance Image Texture" (1998-2002) and COST B21 European project "Physiological modelling of MR Image formation" by researchers from the Institute of Electronics, Technical University of Lodz (TUL), Poland (14, 15).

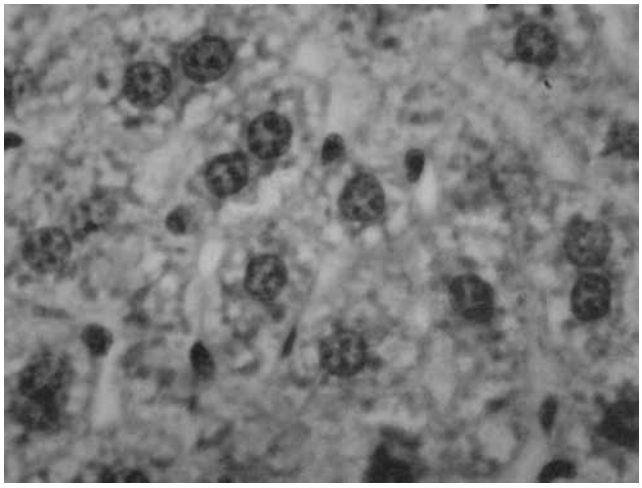

Figure 1. An example of gray-scale digital micrograph of hepatic tissue in 8-bit format suitable for GLCM analysis.

During the GLCM analysis, the resolution units of the grayscale ROI are associated with gray level values after which a complex second-order statistical and mathematical analysis is performed taking into account the value pairs. Similarly to our previous works (11, 16), we calculated the GLCM features of inverse difference moment (IDM), angular second moment (ASM), in line with the following formulae:

$$ASM = \sum_i \sum_j \{p(i,j)\}^2$$

$$IDM = \sum_i \sum_j \frac{1}{1+(i-j)^2} p(i,j)$$

In GLCM algorithm, the p(i,j) represents the (i,j)th entry of the normalized co-occurrence matrix, whereas the mean and the standard deviation respectively of rows x and y are marked as μ and σ. Inverse difference moment and angular second moment correspond to the levels of local textural homogeneity and uniformity, respectively. GLCM Contrast (CON), GLCM Correlation (COR), and GLCM Sum Variance (SVAR) were calculated as:

$$CON = \sum_i \sum_j (i-j)^k P_d[i,j]^n$$

$$COR = \frac{\Sigma_i \Sigma_j (ij) p(i,j) - \mu_x \mu_y}{\sigma_x \sigma_y}$$

$$SVAR = \sum \left[i - \sum i p_{x-y}(i) \right]^2$$

Textural contrast and correlation are the degree of variation of gray level intensities and the linear dependencies of gray values, respectively, while the variance represents the degree of dispersion of the distribution of gray values (17-19).

Initial statistical analysis was done in SPSS software (v.25.0; IBM, Chicago, IL). The Random Forest (RF) classifier, a widely accepted ensemble learning method was used to train and test the model in Scikit-learn library for Python programming language (20). In this model, a multitude of decision trees are constructed based on a random subset of features at each split. We used all 5 GLCM indicators as input data whereas the designated target was the class of the ROI, in terms of its affiliation to the experimental or control group. This approach and Python code were previously developed on yeast cell model as a part of the SensoFracTW project supported by the Science Fund of the Republic of Serbia. Approximately 80% of the data were used for training while the remaining data were used for testing and calculation of classification accuracy. The discriminatory power of the model was determined through receiver operating characteristics analysis. An alternative, decision tree model, based on the classification and regression tree (CART) classifier was also created in Scikit-learn with similar settings.

RESULTS

The average values for GLCM angular second moment were 0.014±0.002 and 0.016±0.003 for the experimental and control ROIs, respectively ($p<0.01$). The mean values of inverse difference moment were also significantly reduced in the experimental group (0.767±0.012) compared to controls (0.770±0.0123, $p<0.01$). On the other hand, the values of textural correlation significantly increased in hepatic tissue exposed to iron oxide nanoparticles (0.9953±0.0008 compared to 0.9951±0.0009, $p<0.05$). A similar increase was observed in the values of GLCM sum variance which in the experimental group equaled 284.8±49.6 and in controls 277.1±56.9 ($p<0.01$). No significant difference was detected in the values of textural contrast (0.65±0.06 in the experimental group, 0.64±0.06 in controls, $p>0.05$).

The traditional classification and regression tree model was successfully developed in Scikit-learn Python library in Google Collaboratory project platform. The estimated accuracy of the CART model was solid, although

not outstanding 68.67% (**Figure 2**). The area under the roc curve for this approach equaled 0.75 indicating acceptable discriminatory power in separating ROIs from treated and control hepatic tissue. When feature importance analysis was performed, it was concluded that angular second moment was the most contributing input parameter with the importance value of 0.74.

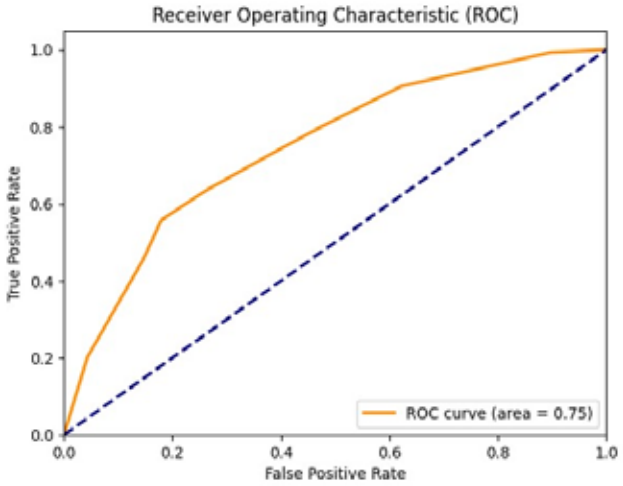

Figure 2. Receiver operating characteristic curve for CART machine learning model.

Random forest model had classification accuracy of 73.67%, after hyperparameter tuning. This accuracy was achieved grid search instantiation with multiple-fold cross-validation. The area under the receiver operating characteristics curve for this approach equaled 0.81 which may be considered as relatively good discriminatory power in terms of separation of ROIs associated with damaged and control tissue (**Figure 3**). As with the CART model, when feature importance analysis was performed, angular second moment was shown to be the most contributing input parameter although with much lower importance score of 0.42. The F1 score for the model equaled 0.74 meaning that the model had fairly good, but not perfect, precision and recall.

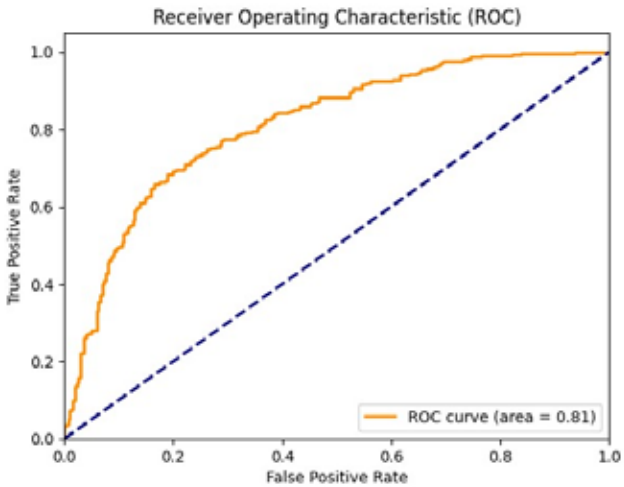

Figure 3. Receiver operating characteristic curve for Random Forest machine learning model

DISCUSSION

In this paper we showed that it was possible to develop a machine learning model based on the random forest algorithm capable of differentiating between ROIs of normal hepatic tissue and the tissue exposed to iron oxide nanoparticles. The application of the textural indicators of the gray level co-occurrence matrix as input data for model training was demonstrated to be a potentially useful approach for the assessment of hepatic structure in these experimental conditions. The random forest model outperformed the alternative classification and regression tree classifier in terms of the classification accuracy and the estimated area under the receiver operating characteristic curve. The results of this study as well as the developed computer code for machine learning models serve as the foundation for further exploration of artificial intelligence strategies in pathophysiology and pathology research.

This is not the first research where gray level co-occurrence matrix features were applied as input data for decision tree model training. Recently, Davidovic et al. (2022) used similar computation approach to detect subtle changes in cell nuclei associated with exposure to sublethal dose of ethanol. On a cell experimental model angular second model, inverse difference moment, GLCM contrast, GLCM correlation and variance were considered as input for the development of not only random trees but also models based on binomial logistic regression and multilayer perceptron neural network (21, 22). All models showed relatively solid parameters of performance although the perceptron slightly outperformed the alternatives in terms of classification accuracy. Unfortunately, due to significant differences in methodology for GLCM calculation, as well as in techniques for micrograph creation and model training, the results are not comparable to the ones from our current study.

Random forest itself, created in scikit-learn Python library trained using GLCM and other data was demonstrated to be a potentially useful artificial intelligence approach in yet another recently published in vitro research (11). Here, cells were exposed to hyperosmotic environment and discrete changes in nuclear structure were evaluated by calculating the values of inverse different moment, angular second moment, contrast, correlation and variance, similarly to our current study. However, unlike the current study, the authors performed additional calculations of nuclear fractal dimension as an indirect indicator of structural complexity and level of detail. Also, a feature of discrete wavelet transform (wavelet coefficient energy) was calculated to further explain variations in structural heterogeneity of the nuclear structures (11). Finally, all the calculated features were used as input data while the target was the class/status of the cells in regard to the treatment. Random forest achieved a solid classification accuracy of 79.8% which was significantly higher compared to the alternative support vector machine approach.

Additional future work and applications related to our data might include research on further optimization of model hyperparameters which could additionally increase both classification accuracy and discriminatory power. The model should be compared with alternative approaches such as the ones based on multilayer perceptron neural networks, support vector machines and k-nearest neighbors algorithm (3). One should also consider alternative ensemble decision tree models that use sequentially – built trees fitted to residual errors (negative gradient) of the loss function such as gradient boosting trees. These approaches might in some cases be more powerful than random forest, especially when applied to the features of two-dimensional microscopic data. Additionally, all the abovementioned models should be created taking into account data obtained from different experimental protocols that vary in histological staining, magnification, micrograph dimensions and resolution, as well as settings during the micrograph acquisition. The best performing model could be deployed as a part of a computer application using a Python module for serializing and deserializing objects, the best example probably being the well-known "pickle" library. The application could be tested on other datasets obtained from experiments that used alternative iron compounds and could serv as an addition to the current pathohistology and pathophysiology protocols.

The limitation of our research includes several methodological challenges that need to be commented on in this paper. First, the methodology of machine learning, including the applied random forest approach has its own limitations regarding interpretability and reproducibility. Unlike individual decision trees, random forest due to its complexity suffers from the relative lack of interpretability, and inability of a researcher to precisely demonstrate the inner workings of the model. This phenomenon in machine learning is called "black box" and is not specific to random forests (1). Second, in our research, the model was trained on the GLCM data obtained from individual regions of interest of hepatic tissue. This approach where ROIs are considered as statistical units of measurement has its weaknesses in terms of not being certain that the model can be successfully applied in other conditions. For example, the level of classification accuracy in future models designed for differentiation of animals instead of ROIs, may be much lower, even if researchers' overcome challenges related to dataset size for model training. Finally, GLCM as a computational signal analysis technique may sometimes yield results with significant variability depending on the applied methodology and experimental protocol. For example, variations in staining protocol, micrograph acquisition settings, software platform used for GLCM computations and many other factors may influence the values of GLCM features. Subsequently, this may lower the reproducibility of the trained machine learning model and render it less applicable in future research.

CONCLUSION

In conclusion, our study demonstrates that it is possible to create a supervised machine learning model based on random forest that uses GLCM data to differentiate hepatic tissue based on its exposure to iron oxide nanoparticles. The random forest model is superior to the alternative CART decision tree approach in terms of its classification accuracy and discriminatory power. The data obtained as a result of this study may be utilized for further development of artificial intelligence computation systems for identification of physiological and pathophysiological changes in hepatic tissue. The results are also a starting point for further research on the automation of histopathological analysis of liver tissue exposed to external toxic agents.

Conflict of interest

None to declare.

Author contributions

Jovana Paunović Pantić and Igor Pantić contributed the conception and design of the paper, the acquisition, analysis, and interpretation of data and preparation of the draft of the manuscript. Danijela Vučević, Tatjana Radosavljević, and Svetlana Valjarević contributed to the conception and design of the paper and preparation of the draft of the manuscript.

Ethical approval

The study was a part of the PhD thesis research which received approval from both the Ethics commission for the protection and welfare of experimental animals of the University of Belgrade, Faculty of Medicine (approval number 229/2) and the Ministry of Agriculture, Forestry, and Water Management of the Republic of Serbia - Veterinary Division (approval number 323-07-07783/2020-05). The procedures that followed were in line with the Universal Declaration of Animal Welfare (WSPA, London, 2000), the European Convention on the Protection of Vertebrates Used for Experimental and Other Scientific Reasons (1998), and various other domestic and international guidelines promoting responsible and compassionate treatment of experimental animals.

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RAZVOJ MODELA MAŠINSKOG UČENjA ZASNOVANOG NA ALGORITMU SLUČAJNIH ŠUMA ZA DETEKCIJU PROMENA U TKIVU JETRE NAKON IZLAGANjA ČESTICAMA OKSIDA GVOŽĐA

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Sažetak

Uvod/Cilj: Cilj naše studije je bio razvoj modela mašinskog učenja zasnovanog na algoritmu slučajnih šuma, koji koristeći teksturalne podatke iz digitalnih mikrografa tkiva jetre, ima sposobnost da napravi razliku između normalnog tkiva jetre, i oštećenog tkiva izloženog česticama oksida gvožđa.

Materijal i metode: Regioni interesa u okviru digitalnih mikrografa tkiva jetre, dobijenih od miševa koji su tretirani česticama oksida gvožđa i miševa kontrolne grupe, analizirani su korišćenjem metode matriksa simultanog pojavljivanja sivih vrednosti („GLCM" metoda). Dobijeni teksturalni parametri su korišćeni kao ulazni podaci za treniranje i testiranje modela slučajnih šuma u okviru biblioteke "Scikit-learn" u programskom jeziku Python. Dodatno, razvijen je i konvencionalni model mašinskog učenja drva odluke, zasnovan na algoritmu drva klasifikacije i regresije („CART" algoritam).

Rezultati: Model slučajnih šuma nadmašio je alternativni pristup CART drva odluke u pogledu tačnosti klasifikacije, predviđajući ciljnu klasu uzorka u 73,67% instanci u validacionom skupu podataka. Površina ispod krive karakteristika operatera bila je 0,81, ukazujući na relativno dobru diskriminišuću moć modela. F1 skor modela je iznosio 0,74 što pokazuje dobru, mada ne savršenu preciznost i povrat informacija.

Zaključak: Podaci dobijeni iz ove studije mogu se iskoristiti za dalji razvoj sistema zasnovanih na veštačkoj inteligenciji za identifikaciju fizioloških i patofizioloških promena u tkivu jetre. Rezultati takođe služe kao početna tačka za dodatna istraživanja vezana za automatizaciju histopatološke analize jetrenog tkiva izloženog eksternim toksičnim agensima.

Ključne reči: veštačka inteligencija, mašinsko učenje, oksid gvožđa, jetra

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CASE REPORT

Multiple basal cell carcinomas following x-ray treatment of tinea capitis in childhood: a case report and a literature review

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Summary

Introduction: Basal cell carcinoma (BCC) is the most frequent skin cancer with a rising incidence worldwide. Predilection sites are the head and the neck in 80-85% of cases. Leading risk factors are sun exposure and ionizing radiation. In the middle of the 20th century, radiation-induced epilation was used as an efficient treatment method for tinea capitis.

Patient review: We report a case of an 80-year-old man with a 16-year history of the succesive occurrence of multiple BCCs on the scalp, face, and neck. He also had the history of tinea capitis treated with radiotherapy during childhood. At presentation during clinical and dermoscopic examination approximately 25 BCCs were observed, predominantly on the scalp. The most common BCC lesion was the nodular subtype, followed by the pigmented and superficial subtypes. Histopathological examination confirmed the diagnosis of BCC in multiple lesions and one squamous cell carcinoma (SCC). In personal history, the most important comorbidity was hairy cell leukemia. The therapeutic approach included surgery and 5-fluorouracil 5% cream.

Conclusion: Radiation-induced epilation was used as an efficient method for treating tinea capitis. One of its side effects was the appearance of BCC in radiation exposed areas. Surgical excision is the gold standard for BCC treatment. Also, 5-fluorouracil 5% cream may be a good option for patients with multiple BCCs. We present satisfactory results after combined treatment in our patient.

Key words: basal cell carcinoma, ionizing radiation, radiotherapy, tinea capitis, treatment

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INTRODUCTION:

Basal cell carcinoma (BCC) is the most frequent skin cancer with a rising incidence worldwide. Most cases are located mainly on sun-exposed areas such as the head and the neck (80-85%) and are locally invasive, indolent and characterized by slow growth and rare metastases (1). In the middle of the 20th century, radiation-induced epilation was used as an efficient treatment for tinea capitis (2, 3). Radiotherapy of the scalp during childhood is a widely recognized risk factor for BCC (4) and it is the subject of numerous published studies (5-15). We present a patient with multiple BCCs, solitary squamous cell carcinoma (SCC) and hairy cell leukemia.

CASE REPORT

We report a case of an 80-year-old male with a 16-year history of the successive occurrence of multiple BCCs on the scalp, face, and neck. On admission during clinical **(Figure 1a and Figure 1b)** and dermoscopic examination approximately twenty-five BCCs were observed. The most common type of BCC was the nodular type **(Figure 2)**, followed by pigmented and superficial subtypes **(Figure 3a and Figure 3b)**. On the left auricle there was one nodular lesion repetitively bleeding on gentle touch **(Figure 4)**. In personal history he reported radiotherapy for scalp tinea at the age of 12, hairy cell leukemia at the age of 59, hypertension, arrhythmia and benign prostatic hyperplasia. Multiple BCCs started to appear at the age of 64, which is 52 years after radiation exposure. Hairy cell leukemia had been treated with purine analogue cladribine (2-chlorodeoxyadenosine) for 19 years until remission. Family history was negative for skin or other malignancies.

Figure 1. Multiple BCCs on the head and neck and SCC on the left auricle

Figure 2. Dermoscopic findings: comma like vessels, haemorrhage, hypopigmented areas and telangiectasias.

Routine laboratory tests, including complete blood count, biochemical analysis, tumor markers and urinalysis, were within normal limits. Histopathological examination confirmed the diagnosis of BCC in multiple lesions and SCC in a nodular lesion on the left auricle. Neck ultrasonography showed no pathological findings.

The treatment included surgical excision for multiple BCCs (over 15 times) and solitary SCC on the left auricle, and the topical treatment with 5-fluorouracil 5% cream for 6 weeks for inoperable BCCs **(Figure 5a and Figure 5b)**. A great compliance with the patient was achieved.

DISCUSSION

BCC is the most common skin cancer with an increasing incidence worldwide (1). Treatment comprises surgical excision as the gold standard, topical therapy with 5-fluorouracil 5% or 5% imiquimod cream and sonic hedgehog inhibitors vismodegib and sonedegib for locally advanced

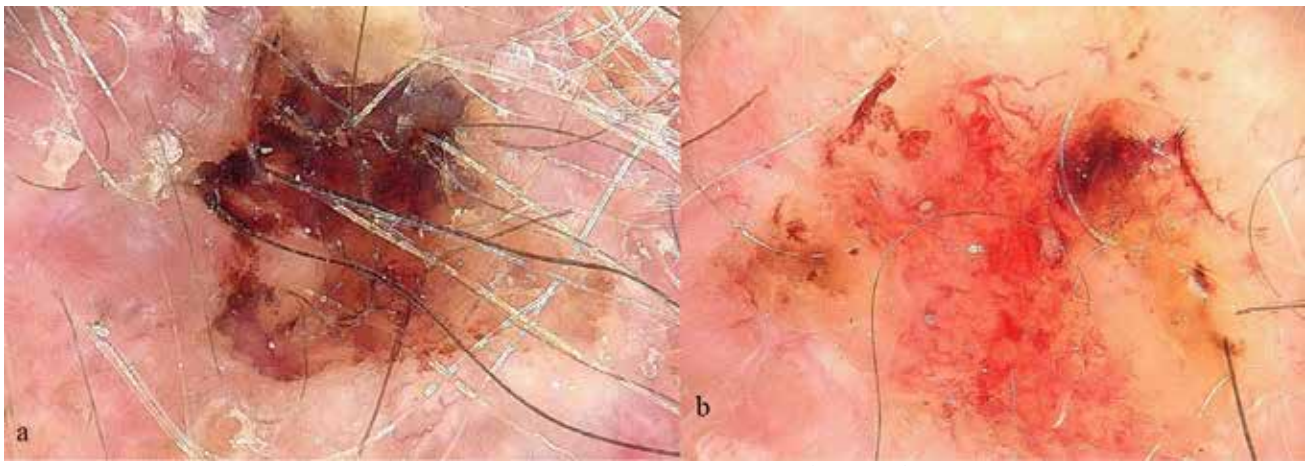

Figure 3. Dermoscopic findings: specks of brown and grey pigment, focal ulceration (left photo); milia, disordered and streaky crystalline structures and white rim around central ulceration (right photo).

and metastatic BCC (6). Exposure to UV radiation and previous ionizing radiation have been recognized as main risk factors for BCC in literature (5, 7, 8).

Figure 4. Multiple BCCs on the head and solitary SCC on the left auricle.

Albert et al. in 1968 (9) reported the first study of long term effects after x-ray treatment for tinea capitis in childhood. High prevalence of multiple BCCs was reported. The most common histological type was nodular, like in our patient. A similar study was published by Maalej et al. (10). In this study, BCCs and SCCs were the most common radiation-induced skin cancers, children were between 6 and 18 years of age and skin cancers started to appear 22 to 50 years after radiation exposure. In reviewed literature, there are data on an inverse relationship between the occurrence of BCC and age when the irradiation occurred. (8, 11). Boaventura et al. also found that there was a greater risk of developing BCC if the radiation exposure occurred at a younger age (12), which is similar to our patient who was irradiated in childhood.

Shore et al. reported comparative study in which 3,604 children participated and were followed up to determine the incidence of cancer after a follow-up period of up to 50 years. The children were divided into two groups, the first containing patients who were treated with X-ray therapy and the second containing those who were treated only with topical medications (13). In the irradiated group, about 40% of patients developed multiple BCCs and only 0.31% SCCs. This study also indicated the importance of skin phototype and sensitivity to UV radiation as well as that children irradiated at a young age had the highest BCC risk.

Mseddi et al. (14) reported 33 patients with diagnosed scalp BCC who were treated in childhood with radiation therapy due to tinea capitis. The median age at the moment of the irradiation treatment was 9 years. The latency period between the received X- ray therapy and the onset of BCC ranged between 21 and 51 years, and in our patient, the first lesion appeared after 52 years. Both clinically and histologically, nodular BCC was the predominant tumor subtype, which is the case in our patient as well.

Another retrospective study from Iran evaluated the difference between previously irradiated and nonirradiated group of patients with BCC tumors during a 10-year follow-up (15). Patients with a history of childhood therapeutic radiation had a more aggressive form of BCC, and needed more extensive surgical treatment, while the recurrence rate of BCC was significantly higher.

In the reviewed literature, BCC was described in the largest number of patients, as a result of radiation exposure of the head and neck. However, there are also data according to which patients developed more malignant diseases (10, 16), like in the case of our patient who developed three malignancies: BCC, SCC, and hairy cell leukemia. The previously mentioned study by Shore et al. also assessed the risk of tumors and malignancies following childhood irradiation treatment for ringworm of the scalp. They found an apparent excess of hematological malignancies in the irradiated group. Hairy cell leukemia was diagnosed in one patient (16).

Figure 5. Scars and residual pigmentation after combined treatment.

CONCLUSION

Radiation-induced epilation as a treatment option for tinea capitis showed to be associated with multiple side effect such as BCC and SCC development. Since sonic hedgehog inhibitors may be promoters of cutaneous SCC (17, 18), they were not considered as a therapeutic option in our patient. Nevertheless, we achieved satisfactory treatment results with occasional surgical excisions of solitary lesions and topical treatment with 5-fluorouracil 5% for superficial BCCs.

Author contribution

All listed authors contributed equally to the conception of the work, the interpretation of data, preparing the draft of the manuscript and the interpretation of the revised version.

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MULTIPLI BAZOCELULARNI KARCINOMI NAKON PRIMENE RADIOTERAPIJE U LEČENJU TINEA CAPITIS U DETINJSTVU: PRIKAZ SLUČAJA I PREGLED LITERATURE

Jelena Cakić [1], Branislav Lekić [1,2], Iva Maširević Mudrić [1], Marija Tomanović [1], Svetlana Popadić [1,2]

Sažetak

Uvod: Bazocelularni karcinom (BCK) je najčešći karcinom kože sa rastućom incidencijom širom sveta. Predilekciona mesta su glava i vrat u 80-85% slučajeva. Vodeći faktori rizika za nastanak ovog karcinoma su izlaganje UV zračenju i jonizujuće zračenje. Polovinom 20. veka, epilacija kapilicijuma izazvana zračenjem korišćena je kao efikasan metod u lečenju gljivičnih infekcija kapilicijuma.

Prikaz pacijenta: Predstavljamo slučaj osamdesetogodišnjeg muškarca sa šesnaestogodišnjom istorijom uzastopne pojave multiplih BCK na kapilicijumu, licu i vratu. Zbog gljivične infekcije kapilicijuma u detinjstvu lečen je radioterapijom. Kliničkim i dermoskopskim pregledom uočeno je približno 25 BCK, pretežno na kapilicijmu. Najčešći tip BCK je bio nodularni, potom pigmentni i superficijalni tip. Histopatološki je potvrđena dijagnoza BCK kod višestrukih lezija i jedan skvamocelularni karcinom (SCK). U ličnoj anamnezi kao najvažniji komorbiditet izdvaja se leukemija vlasastih ćelija. Terapijski pristup zasnivao se na hirurškom lečenju i primeni 5-fluorouracil 5% krema.

Zaključak: Epilacija kapilicijuma izazvana zračenjem korišćena je kao efikasan metod za lečenje gljivičnih infekcija kapilicijuma. Jedan od neželjenih efekata bila je pojava BCK u područjima izloženim zračenju. Iako je hirurška ekscizija zlatni standard za lečenje BCK, primena 5% 5-fluorouracil krema može biti dobra opcija za pacijente sa multiplim BCK. Predstavljamo zadovoljavajuće rezultate nakon primenjene kombinovane terapije kod našeg pacijenta.

Ključne reči: bazocelularni karcinom, jonizujuće zračenje, radioterapija, tinea capitis, lečenje

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ORIGINAL ARTICLE

The role of HE4 protein expression in relation to clinicopathological features of renal cell tumors

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Summary

Introduction: Widespread routine ultrasound diagnostics enables early detection of kidney tumors. However, due to nonspecific symptomatology and signs that usually appear as the disease progresses, there are still patients whose diagnosis is made only in advanced stages of the disease.

Aim: Our aim was to investigate the correlation of human epididymis protein 4 (HE4) expression, including the intensity and localization of HE4 positivity, with the clinical and pathohistological characteristics of kidney tumors.

Material and Methods: The study included 96 kidney tumors diagnosed between 2010 and 2013 at the Institute of Pathology in Belgrade. Anti-HE4 antibodies were used for immunohistochemical analysis. Demographic, clinical, and pathohistological characteristics were examined in relation to HE4 expression.

Results: No correlation was observed between HE4 expression in kidney tumors and patients' gender and the nuclear grade of tumors. However, HE4 expression was significantly more frequent in larger tumors, specifically in T3 and T4 tumors, compared to T1 and T2 tumors (p=0.009; p=0.006, respectively). No correlation was observed between HE4 expression and the pathohistological type of kidney tumors, but it is important to emphasize that membrane expression of HE4, unlike most renal cell carcinomas, was not observed in oncocytomas.

Conclusion: It's possible that HE4 plays a role in progression of kidney tumor growth. Membrane expression of HE4 could be used as a new parameter in differentiating renal cell carcinomas from oncocytomas.

Keywords: renal cell carcinoma, oncocytoma, human epididymis protein 4, HE4, pathohistological characteristics

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INTRODUCTION

According to their biological behavior, tumors of the kidney with renal cell origin can be either benign or malignant. Kidney cancers in adult population occur with a prevalence of about 2%, and they are more frequent in males (2:1) (1, 2). Renal cell carcinoma (RCC) is the most common type of kidney tumor and ranks eighth in terms of its incidence among adult malignancies (3,4). There are several histopathological subtypes of RCC, which occur with variable frequency, the most common being clear cell carcinoma (80-90%), followed by papillary (10-15%), chromophobe (4-5%), and carcinoma of the collecting ducts (1%) (2). These histopathological subtypes have different origins, genetics, morphology, and biological behavior (2). Oncocytoma is a benign tumor of renal cell origin which occurs with a frequency of 3-5% (5). Enhancing our comprehension of the molecular pathways involved in the development and progression of kidney tumors holds a potential to drive the formulation of innovative approaches for early detection and treatment. Human epididymis protein 4 (HE4) is encoded by the gene on chromosome 20q12-13.1 and it was first identified in the epithelium of the distal epididymis (6, 7). Initially, it was believed that HE4 played a role in maturation of the sperm and intrinsic immunity (7, 8). Clinical research in the past decade has shown that HE4 express in other organs, as well, including the female reproductive system, breast tissue, and kidneys, as well as in certain regions of the respiratory tract and nasopharynx (9-11). HE4 synthesis occurs within the endoplasmic reticulum and Golgi apparatus. Subsequently, the protein is secreted via exocytosis into the extracellular space, where it functions as a protease inhibitor (7). HE4 has been recently identified as a potential serum biomarker for ovarian carcinoma, either alone or in combination with CA125 (11-15), also elevated serum levels of HE4 protein have been reported in renal fibrosis (16,17). Interestingly, researchers have shown that systemic administration of HE4-neutralizing antibodies inhibits the progression of renal fibrosis in an animal model (16,17). Considering this, recent studies have shown that HE4 could be a diagnostic marker for ovarian tumors, lung adenocarcinoma, breast, urothelial and pancreatic carcinomas (18-23). Our aim was to investigate the correlation between the expression of HE4 protein, including the intensity and localization of HE4 positivity, with clinical and pathohistological characteristics of renal tumors.

MATERIALS AND METHODS

The study conducted at the Institute of Pathology, Faculty of Medicine, University of Belgrade, between 2010 and 2013, included 96 renal tumors. These comprised 66 clear cell RCCs, 12 papillary RCCs, 7 chromophobe RCCs, 4 multilocular cystic RCCs, 2 collecting duct carcinomas (Bellini), and 5 oncocytomas. Tissue microarray cylinders were obtained from paraffin-embedded renal tumor tissue samples. The sampling process involved triplicate collection from the region of interest in paraffin blocks of the tumor. A hollow medical needle with 0.6 mm diameter was used for this purpose. Taken tissue cylinders were subsequently inserted into the paraffin block and precisely arranged as a series. Using a microtome, the paraffin blocks of the tissue microarray were sliced to the thickness of 5 μm and placed on slides, for subsequent immunohistochemical analysis.

Immunohistochemistry

Immunohistochemistry was performed on tissue microarray plates. Having been deparaffinized in xylol and hydration, the plates were inserted in the citrate buffer (pH 6.0) and exposed to microwave irradiation at 400W for 20 min. Blockage of peroxidase activity was performed with 1% BSA (Bovine Serum Albumin). After the extraction of the antigen, incubation with the primary HE4 antibody (1:40, ab24480, Abcam, UK) was performed for 1 hour. EnVisionTM (DAKO, Denmark) was used to visualize the antigen-antibody reaction with a 3,3'-diaminobenzidine (DAB), and consequently contrasting with hematoxylin (Merc, USA). Negative controls were established by excluding the primary antibody during the immunohistochemistry procedure. As for the positive control, normal human epididymis tissue was used. The slides were examined using a BX53 light microscope with a DP12CCD camera (Olympus).

Statistical analysis

Statistical analysis was performed using IBM SPSS software, version 26.0. Demographic, clinical and pathological characteristics of renal tumors (patient's gender, tumor size, tumor type, nuclear grade and TNM stage of disease) were examined concerning the presence, intensity and localization of the expression of HE4 protein.

RESULTS

In the analysis of 96 cases of kidney tumors, no significant difference was observed in the distribution of HE4-expression in relation to patients' gender. However, we did observe variability in the expression of HE4 among different histopathological types of kidney tumors. The positivity of HE4 was detected in approximately 76% of clear cell RCC and 90% of papillary RCC type 2. In all other tumor subtypes, the expression of HE4 was present in 100% of cases. Upon statistical analysis, no significant correlation was found between the frequency of HE4 expression and nuclear grade (p=0.427).

Table 1. Pathohistological characteristics of kidney tumors and HE4 expression

Pathohistological characteristics		HE4 protein expression n (%)		p
		Absent	Present	
Tumor type	*Clear cell RCC*	16 (24.2%)	50 (75.8%)	#
	Papillary RCC, type 1	0 (0.0%)	2 (100.0%)	
	Papillary RCC, type 2	1 (10.0%)	9 (90.0%)	
	Multilocular cystic RCC	0 (0.0%)	4 (100.0%)	
	Chromophobe RCC	0 (0.0%)	7 (100.0%)	
	Carcinoma of the collecting ducts-Bellini	0 (0.0%)	2 (100.0%)	
	Oncocytoma	0 (0.0%)	5 (100.0%)	
Nuclear grade (NG)	*NG I, NG II*	8 (16.0%)	42 (84.0%)	χ^2=0.710 p=0.427
	NG III, NG IV	9 (23.1%)	30 (76.9%)	
T stage	*T1, T2*	12 (28.6%)	30 (71.4%)	χ^2=7.507 p=0.006*
	T3, T4	2 (5.3%)	36 (94.7%)	
N stage	*N0*	1 (11.1%)	8 (88.9%)	#
	N1	0 (0.0%)	2 (100.0%)	
M stage	*M0*	0 (0.0%)	1 (100.0%)	#
	M1	0 (0.0%)	2 (100.0%)	

In contrast to T1 and T2 tumors, where the expression of HE4 was positive in 71% of cases, higher stage tumors (T3 and T4) were significantly more likely to express HE4 (95%), with a p=0.006. **(Table 1)**.

Given the fact that lymph nodes were submitted only in 11 patients, an adequate statistical analysis was not possible. However, it was observed that tumors without regional lymph node metastases (N0) exhibited HE4 expression in 89% of cases, while all tumors with regional lymph node metastases (N1) showed HE4 positivity **(Table 1)**. Additionally, information regarding systemic metastases was available for a small number of patients (3 out of 96). All of these three patients had HE4 expression in their tumor tissue. Two out of three patients had systemic metastases (M1) at the time of diagnosis. One patient did not have any metastases (M0) during the examination of the surgical specimen, but they were later detected **(Table 1)**.

There is a trend of increasing HE4 expression intensity with an increase in the average tumor size. The average size of the tumors without HE4 expression was 5.3±1.8 cm, while the tumors with mild, moderate and strong expression of HE4 had the following average dimensions: 6.5±3.1 cm, 6.6±3.6 cm 7.2±5.0 cm, respectively.

Through the analysis of the HE4 protein expression intensity, it was observed that the majority of clear cell RCC (36%), papillary RCC type 2 (40%), and multilocular cystic carcinoma (50%) exhibited a mild expression of HE4. On the other hand, oncocytoma (60%), chromophobe RCC (57%), and collecting duct carcinoma

Table 2. Pathohistological characteristics of kidney tumors and intensity of HE4 expression

Pathohistological characteristics		HE4 protein expression n (%)				p
		Absent	mild	moderate	strong	
Tumor type	*Clear cell RCC*	16 (24.2%)	24 (36.4%)	19 (28.8%)	7 (10.6%)	#
	Papillary RCC, type 1	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	
	Papillary RCC, type 2	1 (10.0%)	4 (40.0%)	3 (30.0%)	2 (20.0%)	
	Multilocular cystic renal cell neoplasm	0 (0.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	
	Chromophobe RCC	0 (0.0%)	1 (14.3%)	4 (57.1%)	2 (28.6%)	
	Carcinoma of the collecting ducts-Bellini	0 (0.0%)	0 (0.0%)	2 (100.0%)	0 (0.0%)	
	Oncocytoma	0 (0.0%)	2 (40.0%)	3 (60.0%)	0 (0.0%)	
Nuclear grade (NG)	*NG I, NG II*	8 (16.0%)	18 (36.0%)	17 (34.0%)	7 (14.0%)	χ^2=0.712 p=0.870
	NG III, NG IV	9 (23.1%)	13 (33.3%)	12 (30.8%)	5 (12.8%)	
T staging	*T1, T2*	12 (28.6%)	11 (26.2%)	11 (26.2%)	8 (19.0%)	χ^2=10.203 p=0.017*
	T3, T4	2 (5.3%)	17 (44.7%)	15 (39.5%)	4 (10.5%)	
N staging	*N0*	1 (11.1%)	4 (44.4%)	3 (33.3%)	1 (11.1%)	#
	N1	0 (0.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)	
M staging	*M0*	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	#
	M1	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	

*- statistically significant results; #- Due to high occurrence of lower expected frequencies, statistical analysis could not be performed; n- number of cases; N0 – regional lymph node involvement; N1- regional lymph node involvement; M0 – without metastases; M1 – present metastases.

Table 3. Pathohistological characteristics of kidney tumors in relation to the localization of HE4 expression

Pathohistological characteristics		Membranous HE4 expression n (%)		p
		Present	Absent	
Tumor type	*Clear cell RCC*	44 (88.0%)	6 (12.0%)	#
	Papillary RCC, type 1	1 (50.0%)	1 (50.0%)	
	Papillary RCC, type 2	4 (44.4%)	5 (55.6%)	
	Multilocular cystic renal cell neoplasm	4 (100.0%)	0 (0.0%)	
	Chromophobe RCC	4 (57.1%)	3 (42.9%)	
	Carcinoma of the collecting ducts-Bellini	1 (50.0%)	1 (50.0%)	
	Oncocytoma	0 (0.0%)	5 (100.0%)	
Nuclear grade (NG)	*NG I, NG II*	36 (85.7%)	6 (14.3%)	χ^2=1.713 p=0.191
	NG III, NG IV	22 (73.3%)	8 (26.7%)	
T staging	*T1, T2*	26 (86.7%)	4 (13.3%)	χ^2=3.654 p=0.059
	T3, T4	24 (66.7%)	12 (33.3%)	
N staging	*N0*	5 (62.5%)	3 (37.5%)	#
	N1	2 (100.0%)	0 (0.0%)	
M staging	*M0*	1 (100.0%)	0 (0.0%)	#
	M1	2 (100.0%)	0 (0.0%)	

(100%) more frequently demonstrated a moderate intensity of HE4 expression. Half of the cases of type 1 papillary RCC demonstrated a weak expression of HE4, while the remaining half showed a moderate expression of HE4 **(Table 2)**.

The expression intensity of HE4 showed no statistically significant difference (p = 0.870) in relation to the nuclear grade of tumors, the majority of tumors with both lower nuclear grades (NG I and II) and higher nuclear grades (NG III and IV) exhibited a weak expression of HE4 (Table 2).

The highest percentage of tumors in the lower T stages of the disease (T1 and T2) exhibited no expression of the HE4 protein. In contrast, in the higher stages (T3 and T4), the expression of HE4 was more frequent, with lower and moderate intensity, and this difference was statistically significant, p = 0.017, **(Table 2)**.

Tumors without regional and systemic metastases typically displayed weak expression of the HE4 protein, whereas tumors with regional and systemic metastases exhibited more intense expression of the HE4 protein **(Table 2)**.

When analyzing the correlation between tumor size and the localization of HE4 expression, it was observed that kidney tumors without membrane expression of HE4 tended to be slightly smaller in size (6.7 ± 4.0) compared to tumors with membrane HE4 positivity (average of 7.1 ± 4.1).

Out of 79 kidney tumors that expressed the HE4 protein, 58 tumors (75%) exhibited a membrane localization of HE4 either alone or in combination with cytoplasmic localization. On the other hand, 21 tumors (25%) showed exclusive cytoplasmic localization of HE4. Notably, all cases of oncocytoma demonstrated exclusive cytoplasmic expression of HE4, whereas the multilocular cystic neoplasm showed exclusive membrane positivity. **(Table 3, Figure 1)**.

The frequency of membrane expression of HE4 was not significantly different between tumors with lower and higher nuclear grade (p=0.191; **Table 3, Figure 1**) or between tumors with lower and higher T stage (p=0.059; **Table 3, Figure 1**). However, it was observed that with an increase in nuclear grade, clear cell RCC demonstrated not only membrane localization of HE4 but it also exhibited cytoplasmic expression, as illustrated in **Figure 1**. In all cases of tumors with regional and systemic metastases, the presence of membrane expression of the HE4 protein was observed. **(Table 3)**.

DISCUSSION

Over the past three decades, there has been an increasing incidence of renal tumors in Europe, the United States (US), and Australia (3, 4). Approximately 270,000 new cases of renal cell carcinoma (RCC) are diagnosed worldwide each year, and approximately 116,000 patients die from the disease (3, 4). Due to its characteristic rarity of early signs, diverse clinical manifestations, and high resistance to conventional treatments such as radiotherapy and chemotherapy, renal cell carcinoma (RCC) presents a significant challenge in terms of early diagnosis and effective treatment. Consequently, numerous clinical and pathological studies have been conducted to identify potential biomarkers that can aid in early diagnosis and facilitate targeted immunomodulatory interventions to inhibit tumor growth. The aim of such research is to improve patient outcomes and develop more personalized approaches to RCC management. Recognition of HE4 as a biomarker of ovarian cancer, as well as the study of Galgano who examined the expression of HE4 in a variety of normal and malignant human tissues, and who expressed the need for further investigation of this protein (11), led us to further examine the expression of HE4 in different histology types of kidney tumors.

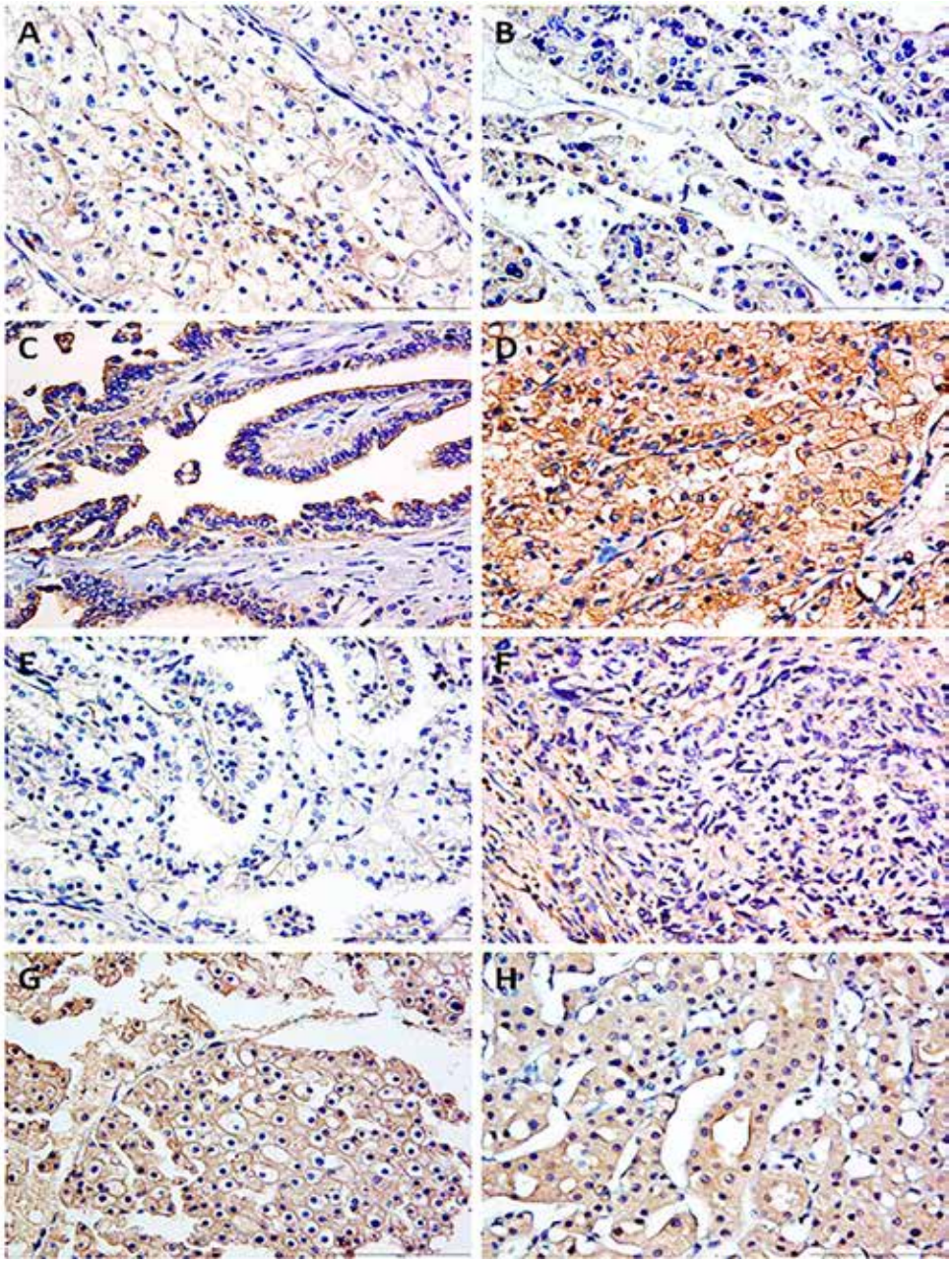

Figure 1. Different patterns of HE4 protein expression in relation to the histopathological tumor type, nuclear grade of the tumor, and biological behavior. **A)** Low nuclear grade clear cell carcinoma - membrane expression of HE4. **B)** High nuclear grade clear cell carcinoma – cytoplasmic and membrane expression of HE4. **C)** Papillary carcinoma type 1 - moderate cytoplasmic and membrane expression of HE4. **D)** Papillary carcinoma type 2 - moderate to strong cytoplasmic and membrane expression of HE4. **E)** Clear cell papillary carcinoma - very weak membrane expression of HE4. **F)** Bellini's collecting duct carcinoma - moderate cytoplasmic and membrane expression of HE4. **G)** Chromophobe carcinoma - moderate cytoplasmic and membrane expression of HE4. **F)** Oncocytoma - moderate expression of HE4 exclusively in the cytoplasm.

Our study yielded compelling results, indicating that 83% of renal tumors expressed the HE4 protein. This finding contrasts with the study conducted by Galgano et al., where a significantly lower percentage of renal tumors (37.5%) demonstrated HE4 immunoreactivity([11).

In our study, we found no association between the patients' gender and the expression of the HE4 protein in kidney tumors. Similarly, other studies measuring serum levels of HE4 concluded that gender did not impact its expression in patients with kidney tumors (15).

Tumor size is an important prognostic factor in RCC, which correlates with a poorer prognosis, higher incidence of metastases and higher mortality (24). US researchers have reported a significant association between serum levels of HE4 and tumor size as well as myometrial invasion in endometrial cancer (25). In our study, we made a novel discovery that larger renal tumors demonstrated a significant expression of HE4. Additionally, we observed that the membrane localization of HE4 positivity was considerably more prevalent in larger tumors compared to cytoplasmic localization. Moreover, we noticed a tendency for the intensity of HE4 expression to amplify as the tumor diameter increased.

The study conducted by Galgano (11) has provided partial examination of HE4 protein expression in different histopathological types of kidney tumors. The

localization of HE4 protein in relation to specific cell compartments has not been assessed in kidney tumors. HE4 is normally expressed within the reproductive system. However, Drapkin observed that ovarian carcinomas secreted this protein abundantly into the bloodstream and urine, allowing for easy detection of its presence and quantity. Consequently, HE4 emerged as a sensitive biomarker for ovarian cancer (10). In our study, we conducted further analysis to investigate the presence, intensity, and localization of the HE4 protein based on the histopathological subtype of renal cell carcinoma (RCC), nuclear grade, as well as the local, regional, and systemic spread of the disease, specifically in relation to the TNM stage.

Clear cell RCC is the most common kidney cancer. Extensive examination of gene expression in many proteins has been carried out (26). Most clear cell RCC expresses HE4 protein, mostly of mild intensity, with present membrane immunoreactivity. Weak expression of HE4 immunoreactivity was also noted by Galgano, but in a much smaller percentage of clear cell RCC (4%), compared to our study (11).

Papillary RCC is recognized as the second most prevalent kidney cancer (27). In our study, HE4 expression was universally present in type 1 clear cell RCC and was found in approximately 90% of type 2 cases, albeit often with mild intensity. This contrasts with the findings of Galgano, who reported HE4 expression in 46% of papillary RCC cases without specifying the subtype. Galgano also observed strong HE4 immunoreactivity in 38% of these papillary RCC cases (11). Interestingly, in our study, none of the type 1 papillary RCC cases exhibited strong HE4 expression, whereas 20% of type 2 cases did.

Multilocular cystic carcinoma is a rare subtype of RCC, which is now considered to be a "multilocular cystic renal cell neoplasia of low malignant potential" (28-30). Distinguishing renal cell carcinoma (RCC) from other histopathologic types, particularly clear cell RCC, is crucial due to the distinct therapeutic strategies involved. This differential diagnosis is essential for determining appropriate treatment approaches (30). Although the difference in HE4 expression compared to other cancers has not been fully understood, it is noteworthy that HE4 is consistently membranously present in all cases of this tumor, albeit with mild intensity.

Differentiating between chromophobe RCC and oncocytoma poses one of the most significant diagnostic challenges in kidney tumor pathology. These tumors have unique biological characteristics that necessitate different therapeutic strategies, making their accurate classification crucial for pathologists (31-34). It is known that histochemistry and the ultrastructure of these two tumors overlap, and the existence of a hybrid tumor which has histological characteristics of both, support the hypothesis of a common precursor of these two tumors (34). Although there are existing markers for the differential diagnosis of oncocytoma and chromophobe renal cell carcinoma (RCC), their sensitivity and specificity have been found to be less than satisfactory. Achieving an accurate diagnosis typically involves a combination of markers. Compared to American authors who have detected 67% oncocytoma and 69% chromophobe RCC with HE4 positivity (11), immunohistochemical analysis of our cases showed the presence of HE4 in all cases of oncocytoma and chromophobe RCC. In our study, for the first time, the localization of HE4 expression was analyzed and we observed a distinct pattern in both oncocytoma and chromophobe renal cell carcinoma (RCC). Specifically, we found that HE4 exhibited cytoplasmic positivity exclusively in oncocytoma, while in 57.1% of chromophobe RCC cases, membrane positivity was also observed. Considering this significant finding, it is conceivable that in the future, HE4 immunohistochemical analysis could be employed as a complementary tool to the routine panel of standard antibodies for the differential diagnosis of oncocytoma and kidney cancer. Since the membranous HE4 immunoreactivity could be an indirect indicator of the secretory activity of the tumor that express HE4, determining the serum level of HE4 might, in some instances, assist in the differentiation of oncocytoma and chromophobe cancer. It is necessary to carry out further testing to determine the correlation between the localization of HE4 positivity and serum values of HE4. The highest percentage of chromophobe RCC and oncocytoma were moderately expressing the HE4, while in his study Galgano observed strong expression of HE4 in both types of tumors (11).

Collecting duct carcinoma is a very rare and aggressive form of RCC (35), so we examined only two cases and noted that in both cases tumor tissue expressed HE4 with moderate intensity. There are multiple similarities between RCC and transitional cell carcinoma. Based on our results and Galgano's results we could point to one more regarding the expression of HE4 protein [36,11].

To date, there is a lack of comprehensive studies investigating the expression of HE4 in kidney tumors and its relation with various clinical and pathological characteristics such as patients' gender, tumor size, histopathologic type, nuclear grade, and TNM tumor stage. However, our study, for the first time, revealed a potential association between HE4 expression in kidney tumors and tumor size.

Despite using the nuclear grade of the tumor as a prognostic indicator, we did not find a significant variation in the frequency and intensity of HE4 expression among tumors with different nuclear grades.

The TNM classification of a tumor is crucial, as it impacts both the therapeutic approach and the prognosis of the disease, while also providing valuable information regarding the possibility of metastasis (37). Our findings indicate that kidney tumors with higher T stages and larger dimensions exhibit a significantly higher frequency of HE4 positivity. Moreover, we observed a nota-

ble correlation between the intensity of HE4 expression and the progression of tumor growth. This might point to a stimulating role of HE4 in the progression of local growth of kidney cancer, as has already been observed in ovarian cancer (38,39).

Some studies show that HE4 can stimulate the invasion and metastasis of various cancers([40). We observed mainly moderate intensity and membrane localization of the HE4 expression in all cases of tumors with both regional and systemic metastases, which might speak in favor of a given hypothesis.

CONCLUSION

Our study showed that renal tumors with larger dimensions and higher stage, more often expressed the HE4 protein, which could play a role primarily in local tumor growth. In our study, a notable distinction was observed between benign tumors, specifically oncocytoma, and malignant tumors. Oncocytoma displayed exclusive cytoplasmic localization of HE4, whereas malignant tumors exhibited variable frequencies of membrane HE4 immunoreactivity. Therefore, the membrane HE4 positivity could be used as a new parameter in the differentiation of kidney cancer and oncocytoma.

Author contributions:

Conception and design: JJ, AM, MZ, GN
Data collection: MT, LS
Writing the article: JJ, AM, MZ, GN
Critical revision of the article: MB, VB
Final approval of the article: JJ, AM, LS, MT, MB, VB, GN, MZ

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ULOGA EKSPRESIJE HUMANOG EPIDIDIMISNOG PROTEINA 4 U ODNOSU NA KLINIČKO-PATOLOŠKE KARAKTERISTIKE TUMORA BUBREGA

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Sažetak

Uvod: Široko rasprostranjena rutinska ultrazvučna dijagnostika omogućava otkrivanje tumora bubrega u početnim stadijumima. Međutim, zbog nespecifične simptomatologije i znakova, koji se javljaju tek kada bolest uznapreduje, još uvek postoje pacijenti kojima se dijagnoza postavlja tek u uznapredovalim fazama bolesti.

Cilj rada: Naš cilj bilo je ispitivanje povezanosti ekspresije humanog epididimisnog proteina 4 (HE4), uključujući intenzitet i lokalizaciju HE4 pozitivnosti, sa kliničkim i patohistološkim karakteristikama tumora bubrega.

Metode: Ispitivanje je uključilo 96 tumora dijagnostikovanih u periodu od 2010. do 2013. godine na Institutu za Patologiju u Beogradu. Korišćeno je anti-HE4 antitelo za imunohistohemijsku analizu. Ispitane su demografske, kliničke i patohistološke karakteristike u odnosu na HE4 ekspresiju.

Rezultati: Nije uočena povezanost HE4 ekspresije u tumorima bubrega sa polom pacijenata i nuklearnim gradusom tumora. Međutim, ekspresija HE4 je bila znatno češća kod većih tumora, odnosno kod tumora u stadijumima T3 i T4, u odnosu na tumore u stadijumima T1 i T2 (p=0,009; p=0,006; respektivno). Povezanost HE4 ekspresije i patohistološkog tipa tumora bubrega nije uočena, ali je u pogledu lokalizacije HE4 imunopozitivnosti najvažnije istaći da membranska ekspresija HE4, za razliku od većine karcinoma bubrega, nije viđena kod onkocitoma.

Zaključak: Moguće je da HE4 ima ulogu u progresiji rasta tumora bubrega. Membranska ekspresija HE4 bi se mogla koristiti kao novi parametar u diferencijaciji karcinoma bubrega i onkocitoma.

Ključne reči: karcinom bubrežnih ćelija, onkocitom, humani epididimisni protein 4, HE4, patohistološke karakteristike

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REVIEW

Preoperative risk assessment in pediatric anesthesia

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Summary

In general pediatric surgical population, the incidence of perioperative 30-day mortality is low. A child's physical status at the time of elective surgery and the presence of comorbidities can notably impact the perioperative risk. In order to provide quality care, preoperative evaluation should be undertaken in a timely and thorough manner.

In preoperative period, it is highly important to be able to identify the patients who are at a higher risk, with the premise that early identification of these patients will allow for additional care to be provided, thereby minimizing possible complications. The main objectives of adequate preoperative assessment are to evaluate patients' readiness for the procedure, optimize patients' health status before surgery, reduce surgical morbidity, and help patients regain their usual functions postoperatively. Routine prescription of additional tests in children should be replaced by selective and rational prescriptions, based on the patients' history and performed clinical evaluation. Surgical and anesthetic complications can have adverse effects on patients' health, and they have also been proven to be associated with increased inpatient and postoperative costs. Each member of the surgical team plays a vital role in the safe care of pediatric patients. Well known risk factors for critical events in the perioperative period in pediatric population are as follows: ASA physical status, age, emergency surgery, and the existence of an underlying disease.

This review highlights potential risks encountered in children and directs preoperative assessment towards selecting essential tests based on identified individual risk factors.

Keywords: anesthesia, pediatrics, preoperative testing, risk factors

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INTRODUCTION

In general pediatric surgical population, the incidence of perioperative 30-day mortality is low (1), but the incidence of perioperative morbidity is still high (2). Most common causes of anesthesia-related cardiac arrests are cardiovascular causes (36%), respiratory causes (27%), medication-related causes (20%), and equipment-related problems (5%) (3). The majority of incidents (80%) occur during the maintenance of anesthesia (4).

A child's physical status at the time of surgery and the presence of comorbidities can notably impact the perioperative risk (5, 6). While the majority of children undergoing anesthesia are of good health, it is of great importance to detect any risk factors that may lead to unanticipated adverse event in the perioperative period. Prior to any procedure that requires the use of analgo-sedation or general anesthesia, preoperative risk assessment is necessary (7, 8). Common practice that implies routine prescription of additional tests in children should be replaced by individualized approach, based on patients' history and clinical examination of every child (9, 10).

Our goal in this review article was to elucidate which pre- and intraoperative patient characteristics in children undergoing surgery are associated with an increased risk of perioperative complications. Knowledge of risk profiles in pediatric anesthesia is a starting point for risk reduction.

PERIOPERATIVE RISK ASSESSMENT TOOLS

There has been a plethora of preoperative risk scores developed in recent years, but most of them have passed only internal validation, and to be applied in clinical settings they need to be externally validated (11). Nowadays, only a few models are used in clinical settings (12). The reasons for the aforementioned are as follows: a relatively low incidence of adverse outcomes in pediatric population, a lack of clear guidance on which score to use, barriers for their application in clinical settings, or the fact that clinicians may find the tools not to be particularly useful in everyday practice.

The American Society of Anesthesiologists Physical Status (ASA PS) classification **(Table 1)** is most widely used, but it has some drawbacks, such as expressed subjectivity in the assessment. It is a six-point risk stratification tool for adults and children. The ASA class is an independent predictor of perioperative morbidity and mortality (13). Another one is the pediatric risk assessment (PRAm) score, which has prognostic value in the perioperative mortality in children who undergo non-cardiac surgeries (14). It is composed of a five-variable objective score derived from the American College of Surgeons (ACS) National Surgical Quality Improvement Program (NSQIP) pediatric database. This scoring system includes the following: an urgent surgical intervention, comorbidities, the characteristic of critical illness, age below12 months, and the presence of malignancy **(Table 2)**. The PRAm score is an objective instrument that can be easily used by clinicians and may potentially improve patient outcomes, but further validation is needed for it to be generally accepted (15).

Table 1. American Society of Anesthesiologists (ASA) classification scoring system

ASA score	Patient preoperative physical status
I	Patient of normal health
II	Patient with a mild systemic disease
III	Patient with a systemic disease that is not incapacitating
IV	Patient with an incapacitating systemic disease that is a constant threat to life
V	Moribund patient who is not expected to survive the following 24h, with or without surgery
VI	A declared brain-dead patient whose organs are being removed for donor purposes

Table 2. Pediatric risk assessment score for predicting postoperative mortality

Variable	Definition	Value
Urgency	An urgent surgical procedure	+1
Comorbidity	The presence of at least one of the following comorbidities: respiratory disease, congenital heart disease, preoperative acute or chronic renal failure, neurologic disease, hematologic disease	+2
Critically ill	The presence of at least one of the following characteristics of critical illness: preoperative mechanical ventilation, inotropic support, preoperative cardiopulmonary resuscitation	+3
Age <12 mo	Age at the time of surgical procedure below 12 months	+4
Malignancy	Surgical procedure in a patient with a neoplasm, with or without chemotherapy	+5

ADDITIONAL ANALYSES

A routinely performed preoperative evaluation of pediatric patients usually includes history taking, physical examination, and laboratory testing. Routine preoperative laboratory analysis for all patients has been questionable over the past several years considering the efforts to decrease medical costs and at the same time preserve the quality of medical care (16). Justified medical reasons for ordering additional tests are as follows: detecting unsuspected but invariable conditions that may alter the operative risk, detecting unsuspected conditions in which interventions may lead to a lower operative risk, and getting baseline results that may be helpful in decision-making during and after surgery.

Routine blood testing in preoperative evaluation of healthy children detects about 2.5 –10% of abnormal re-

sults, but these results infrequently have an impact on the timing of surgery (17). The American Academy of Otolaryngology – Head and Neck surgery recommends screening for coagulation disorders only for patients with a clear medical indication (18). Besides, normal coagulation values do not absolutely rule out a coagulation disorder. Preoperative arterial blood gases are invasive, often difficult to perform in a child who is awake, and do not upgrade the quality of risk assessment. Biochemical analyses and electrolyte status are only justified in children with a history suggesting an underlying disease or who take medications which might affect the water/electrolyte balance, renal or hepatic function. The reason behind performing urine analysis prior to surgery is to detect children with unsuspected renal disease or urinary tract infection (19). Routine chest X-rays rarely reveal clinically important abnormalities which have not been already suggested by a thorough history and physical examination. The American Academy of Pediatrics recommends that there should be no chest X-ray unless there is a clear indication that it will notably impact the perioperative period (20). Spirometry should not be used unselectively, but only where its use could provide additional information that would change perioperative management (21). A cardiac evaluation is recommended in all patients with symptoms suggesting cardiac disease and in all asymptomatic patients with a clinical examination indicating potential for an underlying cardiac disease (22).

PRETERM NEONATES

Neonates and premature neonates have limited physiological reserve, and are at a greater risk of complications with general anesthesia, compared to older children and adults (23). Neonatal surgery, especially in preterm babies, is associated with adverse neurodevelopmental outcomes (24). Prematurely born children are at an increased perioperative risk by the age of three. An anesthesiologist must know that prematurity is a risk factor for acute newborn complications (e.g., intraventricular hemorrhage, bronchopulmonary dysplasia, and retinopathy), as well as complications that continue into childhood (e.g., cognitive or motor delay, delayed growth and development, and heart disease) (7). Also, infants below 60 weeks of postconceptional age are at risk of apnea. It is necessary that infants are monitored until they are apnea-free for at least 12 h postoperatively, before discharge (25).

POSTOPERATIVE NAUSEA AND VOMITING

There is a long list of etiology factors of postoperative nausea and vomiting (PONV) which remain problems in children undergoing anesthesia and surgery (26). We can influence certain etiological factors, and this primarily refers to the anesthesia technique, the use of opioids, pain and anxiety. Opioids and pain-related causes are two of more common causes of PONV which lead to patient and family dissatisfaction (27). Essential elements of a multimodal approach to an adequate anti-emetic and pain therapy are as follows: preoperative risk evaluation and stratification, a multimodal combined anti-emetic prophylaxis, and pain management including opioid-sparing medications and regional anesthesia (28).

PREOPERATIVE FASTING

Adults and children should be encouraged to drink clear fluids up to 2 h before elective surgery; solid food should be prohibited for 6 h, breast milk is safe up to 4 h prior to surgery and other milks are allowed up to 6 h prior to elective surgery (29). Children should not have their surgery cancelled or delayed just because they are chewing a gum or eating a lollipop. There is insufficient evidence for recommending a routine use of antacids, metoclopramide or H2-receptor antagonists before elective surgery.

SPECIFIC DISEASES

Respiratory risk

Respiratory adverse events account for 77% of the total number of incidents (4). Perioperative respiratory adverse events are a main cause of morbidity and mortality and cause up to 30% of perioperative cases of cardiac arrest in pediatric population during anesthesia (30). Well known risk factors for perioperative respiratory adverse events are recent (less than 4 weeks ago) or active upper respiratory tract infection (URTI), primary pulmonary morbidity, a history of snoring, being below 6 years of age, passive smoking, the type of airway device in use, how experienced the anesthesiologist is, and the type of surgery performed.

During the preanesthetic examination, the child should have their mouth wide open to exclude insufficient mouth opening and to extend the neck to exclude cervical spine restrictions (31). Craniofacial malformations, tumors, syndromes, musculoskeletal problems or trauma can lead to difficulties in airway management (32). The incidence of a difficult airway is higher in children under the age of 1 (33).

Respiratory tract infection

One of the most controversial questions in pediatric anesthesia relates to the decision when to proceed with anesthesia and surgery if the child has URTI. Up to 30% of children presenting for elective surgery have an active URTI (34). There is a significant positive correlation

between a URTI and the incidence of perioperative respiratory adverse events, such as cough, laryngospasm, bronchospasm, arterial oxygen desaturation, apnea or breath-holding, hospital readmission, even death (35). Up to 6 weeks following the infection patients with a URTI have modified airway reactivity. In children presenting with signs and symptoms of a lower respiratory tract infection (LRTI) or with fever >38.5C, elective surgery should be postponed for 6 weeks (2).

Asthma

Recent worsening of asthma symptoms, an increase in the need for asthma medications or hospitalization for asthmatic symptoms increase the perioperative risk (36). It is characteristic that bronchial hyperreactivity frequently persists beyond the presence of asthmatic or other respiratory symptoms. Administration of corticosteroids prior to surgery reduces respiratory adverse events if started at least 48 h before surgery, as their onset of action is 6-8 hours upon administration, and they reach their maximal effect in 12 to 36 hours (37).

Bronchopulmonary dysplasia

A suspicion of the presence of bronchopulmonary dysplasia (BPD) should occur if a child was prematurely born and was mechanically ventilated in the neonatal period. Also, during the first day of life these children retain an increased risk of perioperative bronchospasm and oxygen desaturation. Additionally, children with severe BPD may develop right ventricular disfunction that can be worsened by the induction of anesthesia. As they get older, these children may become asymptomatic, but they still have a higher rate of bronchial hyperreactivity (5). Children with a severe form of BPD should be monitored for 24–48 hours upon surgery.

Congenital heart disease

It is of great importance to identify patients with congenital heart disease (CHD) before surgery, even though most murmurs are of functional origin. Children with CHDs are at an increased risk of developing complications during anesthesia, and anesthesia-related cardiac arrest during a non-cardiac surgery is more common (38). Patients with the greatest perioperative risk are infants with a functional single ventricle, suprasystemic pulmonary hypertension (PH), left ventricular outflow tract obstruction and cardiomyopathy (39). Besides, the presence of cardiac failure, PH, arrhythmia, and cyanosis indicate patients with complex problems (22). Possible adverse events during procedures like cardiac catheterization interventions are coronary ischemia, cardiac arrest, low cardiac output, right ventricular failure, pulmonary hypertensive crisis, arrhythmias, cardiac perforation, and tamponade (39).

Pulmonary hypertension

Children with pulmonary hypertension (PH) have a twenty times greater perioperative risk than general pediatric population. Several pediatric studies have reported that the incidence of perioperative cardiac arrest in patients with PH is between 0-5% and perioperative death around 1.5% (40). However, a precise definition of risk stratification for anesthesia in a child with PH is controversial, because of the following: as pediatric PH is not common, the overall incidence of perioperative morbidity and mortality is low, so most pediatric studies are comparatively heterogeneous and various subgroups of pediatric PH have different types of perioperative risk (41).

Acute and chronic kidney disease

Acute kidney injury (AKI) is a common complication of cardiac and non-cardiac surgeries, and it negatively influences short- and long-term outcomes. In children with chronic kidney disease (CKD), preoperative assessment needs to be focused on the presence of cardiorespiratory problems, hypertension, hypo/hypervolemia, electrolyte disbalance and coagulation disorders. CKD increases the risk of postoperative acute kidney injury, major adverse cardiac events, and death. If in preoperative assessment we reveal underlying CKD and other risk factors for AKI, in some situations we may be able to administer preventive therapy and improve the outcomes (42).

Neurologic diseases

Children with neurologic or neuromuscular diseases are at a greater perioperative risk (43). Anticonvulsant therapy should be optimized prior to surgery. The majority of anticonvulsant medications have a long half-time, so missing one dose of anticonvulsants does not significantly decrease the drug blood levels. The function of ventriculoperitoneal shunts should be checked preoperatively and adequate measures should be taken to avoid increased intracranial pressure. In spite of the missing causative connection between malignant hyperthermia and other neuromuscular diseases, triggering agents should be avoided in children presenting with neuromuscular disorders. In case of a positive personal or family history the patient should be tested for malignant hyperthermia.

Obesity

Obesity is associated with pathophysiological changes affecting multiple organ systems, of which most relevant to anesthesiologists are the airway, cardiopulmonary system, the endocrine and hepatic systems. Changes in body composition also affect drug disposition and require awareness of appropriate weight scalars for dosing in obese children (44). All obese children need to

be checked for symptoms of sleep-disordered breathing. Due to the use of sedatives and opioids in anesthesia management, the perioperative period is a time of particularly high risk for patients with obstructive sleep apnea (OSA). Patients with OSA have a higher incidence of postoperative hypoxia, respiratory failure, cardiac events, and intensive care unit transfers than those without OSA (45).

Diabetes mellitus

For optimal management of patients with diabetes mellitus during the perioperative period, pediatric anesthesiologists must carefully consider the pathophysiology of the disease, patient-specific methods of treatment, glycemic control, the type of surgery and surgery timing. The perioperative plan should be developed in consultation with a pediatric endocrinologist (46). The following pediatric issues need to be considered: body size, pubertal development and the ability to tolerate nil per os status. Attention to blood glucose monitoring and insulin therapy is required to maintain normoglycemia and avoid patient distress.

CORTICOSTEROID THERAPY

As the adrenal glands take up to one year to recover entirely following long-term steroid treatment, endocrinologists recommend to substitute it so as to have the discontinuation of corticosteroids for a year. Prior to surgery, children on long-term steroid therapy should receive their daily dose orally or parenterally and an additional dose (stress dose) should be added dependent on the duration and the type of surgery. Patients with long-term inhalation steroids do not need an additional stress dose prior to surgery. Von Ungern-Sternberg et al. recommend their regimen for steroid replacement: for a minor surgery, the first dose of hydrocortisone 50 mg/m^2 intravenously is recommended and should be followed by 12.5 mg/m^2 every 6 h on the day of surgery. For greater surgical stress, a dose of 100 mg/m^2 is recommended followed by 25 mg/m^2 every 6 h on the day of surgery, every 8 h on the first postoperative day, and every 12 h on the second postoperative day. On the third postoperative day, an ordinary treatment dose should be given (47).

ALLERGIES

Patients are exposed to multiple agents that can trigger hypersensitivity reactions in the perioperative period. Perioperative anaphylaxis is an unexpected and life-threatening event, but fortunately its incidence is low (48). The risk group of patients without a prior history of perioperative hypersensitivity reactions need to be recognized in pre-anesthesia examination and if necessary, be referred to an allergologist. The management of perioperative hypersensitivity reactions should be a combined effort between allergologists and anesthesiologists (49).

VACCINATION

Many countries have routine immunization schemes, which include several vaccinations within the first year of life, therefore, every anesthesiologist should check for recent vaccinations in patients of this age. Anesthetic implications of a recent vaccination have not been well investigated, but it is known that anesthesia, stress and trauma are suppressors of the immune system. Also, many anesthetic procedures are performed in this age group without apparent consequences. To reduce the coincidence of the peak systemic reactions to the vaccine with anesthesia and surgery it is reasonable to delay elective surgery for at least 3 days following a killed vaccine or inactivated toxins and 2 weeks following an attenuated vaccine (50).

CHICKENPOX

The question which is necessary to ask is if the child has recently contracted chickenpox or has recently been in contact with a patient suffering from chickenpox. Literature does not provide a unique answer to the question of how long it is necessary to postpone elective surgery after chickenpox; the existing recommendations are mostly based on the basic principles of anesthesiologic care of a child suffering from an infectious disease. According to some opinions, the procedure under general anesthesia should be postponed until the end of the infectious period, which would mean at least 3 months. However, the final decision on the induction of general anesthesia or the postponing of elective surgery is left to the personal assessment of the anesthesiologist in charge (51).

CONCLUSION

Understanding and accurately estimating preoperative risk by accounting for the intrinsic risk of surgical procedures and patient comorbidities will lead to a more comprehensive discussion between patients, families and providers. Also, creating a pediatric preoperative risk assessment checklist would be very useful.

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PREOPERATIVNA PROCENA RIZIKA U PEDIJATRIJSKOJ ANESTEZIJI

Ana Vlajkovic Ivanovic[1], Jelena Pjevalica Dragic[1], Dušica Simić[1,2]

Sažetak

U opštoj pedijatrijskoj populaciji incidencija perioperativnog tridesetodnevnog mortaliteta je niska. Opšte zdravstveno stanje deteta u vreme planirane hirurške intervencije kao i prisustvo komorbiditeta mogu značajno da utiču na perioperativni rizik. U cilju obezbeđivanja adekvatne nege, preoperativna evaluacija se mora sprovesti blagovremeno i temeljno.

U preoperativnom periodu veoma je važno identifikovati pacijente sa povećanim rizikom, uz premisu da rana identifikacija ovih pacijenata omogućava dodatnu evaluaciju i lečenje po potrebi, i na taj način rizik od komplikacija se svodi na minimum. Najvažniji elementi koji čine adekvatnu preoperativnu procenu su sledeći: procena da li je pacijent spreman za hirurišku intervenciju, optimizacija zdravstvenog stanja pred hirurišku intervenciju, smanjenje morbiditeta operativne intervencije i potom vraćanje pacijenta u uobičajeno stanje kao pre hirurške intervencije. Rutinsko propisivanje dodatnih testova kod dece bi trebalo zameniti selektivnim i racionalnim testiranjem, baziranim na istoriji bolesti i kliničkoj proceni. Komplikacije hirurških intervencija i anestezije mogu da imaju nepovoljne posledice po zdravlje pacijenta, ali su takođe povezane sa većim bolničkim kao i postoperativnim troškovima. Svi članovi hirurškog tima imaju vitalne uloge u očuvanju bezbednosti deteta.

Ovaj pregled literature ukazuje na potencijalne rizike sa kojima možemo da se susretnemo u dečjoj populaciji i usmerava preoperativnu procenu prema selektivnim testovima zavisno od individualnih faktora rizika.

Ključne reči: anestezija, pedijatrija, preoperativno testiranje, faktori rizika

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REVIEW

Possibilities of radiotherapy in the treatment of pediatric Hodgkin lymphoma

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Summary

Pediatric Hodgkin lymphoma is a malignant, lymphoproliferative disease of children and adolescents. Radiotherapy is an important form of treatment. The possibility of late toxicity of radiotherapy is a limiting factor in the application of radiotherapy as a treatment modality in pediatric patients.

The technological progress of radiotherapy and the introduction of advanced radiotherapy techniques and proton therapy have improved the precision of radiotherapy and reduced the risk of long-term consequences. These technologies enabled targeted treatment, significantly reducing the exposure of healthy tissues and organs to radiation.

All existing treatment recommendations and conducted cooperative studies have shown that radiation therapy is effective in the treatment of pediatric Hodgkin lymphoma, especially when combined with chemotherapy. On the other hand, there is the possibility of late toxicity to organs in growth and development, as well as the possibility of occurrence of secondary malignancies, which must be carefully considered when deciding on the implementation of radiotherapy.

Radiation therapy represents an important therapeutic approach in the combined treatment of pediatric Hodgkin lymphoma. The combined therapeutic approach has improved treatment results, and advanced radiotherapy techniques will reduce the risk of side effects. Indications for the use of radiotherapy should be carefully evaluated in the treatment of pediatric patients with Hodgkin lymphoma.

Key words: pediatric: Hodgkin lymphoma, radiation therapy, combined treatment, toxicity

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INTRODUCTION

Hodgkin lymphoma represents a group of lymphoproliferative diseases that occur in both children and adults. Although the disease has certain common histological characteristics in these two age groups, it is believed that the biology of the disease is different (1). The distribution of histological subtypes in relation to age, gender distribution, the role of the Epstein-Barr virus (EBV) in pathogenesis, prognosis, and treatment sequelae represent differences between pediatric and adult Hodgkin lymphoma (2). Given that Hodgkin lymphoma is a highly curable disease and that the vast majority of children have long-term survival, Hodgkin lymphoma has become a model for studying long-term adverse effects of radiotherapy and chemotherapy. Radiotherapy with extensive radiation fields and high total doses has resulted in hypoplasia of soft and bone tissues, which is most pronounced in pre-pubertal children (2). In addition, combined chemotherapy with high doses of alkylating agents has been responsible for increased cardiovascular mortality and morbidity (3). The most significant adverse effect of radiation therapy is the occurrence of induced malignancy, which is the most common cause of mortality more than 15 years upon the treatment (4). Considering the aforementioned adverse effects, treatment protocols have aimed for smaller radiation volumes with the use of polychemotherapy (5). Today's treatment of pediatric Hodgkin lymphoma is based on different chemotherapy regimens and more subtle radiotherapy, which involves smaller radiation volumes and lower doses to reduce late therapeutic sequelae. Current protocols for treating Hodgkin lymphoma indicate radiation therapy only in cases of inadequate response to induction chemotherapy, which significantly reduces the use of radiation therapy compared to the historical principle of treating this disease (1).

EPIDEMIOLOGY AND RISK FACTORS

Classical Hodgkin lymphoma represents the most common malignancy in adolescence, occurring most frequently in the age group of 15 and 16 years old, and the eighth most common in the age group of 0 to 14 years old. It rarely occurs in children younger than 4 years old (6). The incidence of Hodgkin lymphoma in the pediatric population is 3.6 per 100,000 for boys and 2.6 for girls. The risk of developing Hodgkin lymphoma at birth, for the period from birth to 39 years old, is 0.14 for males and 0.11 for females (7). According to data from the Institute of Oncology and Radiology of Serbia for the period from 2007 to 2018, 11.6% of the total number of irradiated children had Hodgkin lymphoma, 57.3% were male, and 42.7% were female. Regarding the age distribution of irradiated patients with Hodgkin lymphoma, there were no patients in the group aged 0 to 3 years old, 7.9% were 4 to 8 years old, 15.7% were 8 to 12 years old, and the highest percentage of patients (76.4%) were older than 12 years old (8). Also, EBV is associated with the development of Hodgkin lymphoma in the pediatric population. Latent EBV infection markers were present in 31% of pediatric and adolescent patients, most commonly in the mixed cellularity subtype. In the nodular sclerosis subtype, latent EBV infection was detected in 81% of cases, while in the lymphocyte predominance subtype, there was almost no EBV positivity (5%). Furthermore, the frequency of EBV infection is associated with younger age. The frequency of infection was 73% in children younger than 5 years old, while it was only 17% in adolescents aged 15 years old or older (9). In addition to these factors, immunodeficient states, including AIDS, represent risk factors for the development of Hodgkin lymphoma (10).

PATHOLOGY

The histopathological classification of Hodgkin lymphoma is characterized by the presence of Reed-Sternberg cells (so called "popcorn cells", based on morphological appearance), which represent a clonal population of transformed B lymphocytes of the germinal center of the lymph node, both in pediatric and adult patients. In a small number of cases, Reed-Sternberg cells arise from T lymphocytes (11). According to the World Health Organization (WHO) 2016 classification, there are two immunophenotypes: classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma (12). Classical Hodgkin lymphoma can be further classified into nodular sclerosis type (which is the most common type and occurs in 70-80% of cases), mixed cellularity type (which occurs in 15-20% of cases), lymphocyte-rich type (5%), and lymphocyte-depleted type (1%) (13). Pathological diagnosis of Hodgkin lymphoma is based on morphological features as well as immunohistochemistry staining. Lymphocyte-nodular predominant Hodgkin lymphoma is characterized by the presence of Reed-Stenberg cells in a rich background of small lymphocytes and the absence of other immune cells. IHC of Reed Stenberg cells shows the expression of CD 45 and pan B markers (CD20, CD79-a,OCT-2, PAX 5, BCL 6), small background lymphocytes express also OCT-2 (to a lesser extent than popcorn cells). Nodular sclerosis type is characterized by cellular nodules that are surrounded by collagen bands containing a variable number of Reed-Stenberg cells that are immersed in a mixed inflammatory background. Mixed cellularity subtype is morphologically characterized by the presence of Reed-Sternberg cells with a mixed inflammatory infiltrate comprised of eosinophils, histiocytes, neutrophils and plasma cells. Lymphocyte-depleted type has a similar cell composition as mixed cellularity type, but with more histiocytes and less lymphocytes.

Finally, lymphocyte-rich type is comprised of Reed-Sternberg cells in an abundance of small B lymphocytes. Almost all classic Hodgkin lymphoma subtypes express on IHC staining CD30, but CD15 is expressed in 75% to 85% of cases. (14) In contrast to classical Hodgkin lymphoma, nodular lymphocyte-predominant type is generally CD30 and CD15 negative while CD20 is often expressed (15). It is believed that nodular lymphocyte-predominant type has a better prognosis than classical Hodgkin lymphoma.

PRESENTATION

In 80% of cases, the disease presents with painless lymphadenopathy in the neck or supraclavicular region. Mediastinal lymphadenopathy is present in 75% of adolescents and 25% of younger children. This difference in the incidence of mediastinal lymphadenopathy is explained by a higher frequency of lymphocyte-predominant and mixed-type Hodgkin lymphoma in younger children (15). Although often asymptomatic, mediastinal lymphadenopathy can lead to chest pain, dry cough, shortness of breath, and, in cases of pronounced mediastinal lymphadenopathy, "bulky" mediastinum and superior vena cava syndrome. Isolated subdiaphragmatic lymphadenopathy is rare, occurring in about 5% of cases (16). The release of cytokines from tumor cells can cause nonspecific symptoms such as fever, loss of appetite, itching, fatigue, weakness, and sweating. These symptoms occur in about one quarter of patients (17). Of all the listed nonspecific symptoms, only three symptoms have prognostic significance and play a role in staging the disease and therapy. These are B-symptoms, which include fever above 38.3 degrees Celsius that cannot be explained by other causes, night sweats, and weight loss of more than 10% in the past 6 months before the diagnosis (18).

DIAGNOSIS

In a patient with palpable lymphadenopathy, it is necessary to take a detailed history of the duration of lymphadenopathy, the presence of B symptoms, and the presence of other nonspecific symptoms. During clinical examination, it is important to palpate all available peripheral lymphatics, as well as extralymphatic organs that may be enlarged, such as the liver and the spleen. It is also necessary to perform laboratory tests, including a complete blood count, biochemistry analysis, and erythrocyte sedimentation rate. The gold standard for the pathological diagnosis of Hodgkin lymphoma is the examination of a sample obtained by incisional biopsy of an enlarged lymph node (15).

The initial diagnostic workup for mediastinal lymphadenopathy includes chest radiography and computed tomography (CT), which can evaluate the extent of mediastinal lymphadenopathy and "bulky" mediastinum, which in most protocols is a parameter used to determine the therapeutic approach (14). The current gold standard diagnostic method for active lymphomas is F18-fluorodeoxyglucose (FDG) positron emission tomography (PET-CT) (19). This method is used for both initial staging of the disease and for evaluating the response of the disease to treatment. PET-CT is a significantly more precise method for staging the disease than computed tomography, leading to a change in disease staging, usually to a higher stage in 10% to 30% of cases (20). Although PET-CT is the gold standard in initial diagnosis, contrast-enhanced CT has certain advantages over PET-CT, such as better visualization of lymph node conglomerates, better visualization of intestinal loops, and differentiation from lymph nodes in the abdomen, as well as better assessment of the presence of compression or thrombosis of large mediastinal blood vessels (20).

STAGING OF THE DISEASE

Pediatric Hodgkin lymphoma is staged based on the Ann Arbor classification **(Table 1)** which was developed in 1971 (20) as well as the subsequent Costworld modifications (21). These classifications are based on the location and extent of the disease as well as the presence of nonspecific symptoms. Based on the classification, patients

Table 1. Ann Arbor classification: taken from Kortmann, R.D. and Merchant, T.E. (2018) Pediatric radiation oncology. Cham: Springer.

Stage	Description
Stage I	Involvement of a single lymph node region, or a single lymph node and the surrounding area
Stage II	Involvement of two separate lymph node regions, or a single lymph node or organ and its secondary area. Both affected areas are on the same side of the diaphragm (supra- or infradiaphragmatic).
Stage III	Disease involving regions on both sides of the diaphragm (infra- and supradiaphragmatic)
Stage IV	Diffuse or disseminated disease with the involvement of at least one extralymphatic organ (liver, bone marrow, lungs, and others)
Modifier	**Description**
A	Absence of non-specific "B symptoms"
B	Presence of non-specific "B symptoms", including night sweats, fever higher than 38 °C for 1-2 weeks, and weight loss greater than 10% in the last 6 months
E	Disease is not localized in lymph nodes or has spread from lymph nodes into neighboring tissues
X	Extensive "bulky" disease (extramediastinal mass size of 6-10cm or mediastinal lymphadenopathy > 1/3 of the maximum intrathoracic diameter)

Table 2. Lymphonodal groups

Supradiaphragmatic nodal groups	Infradiaphragmatic nodal groups
Waldeyer's ring (lymphoid ring of nasopharynx and oropharynx)	Spleen
Cervical lymph nodes: occipital, submental, preauricular, submandibular, internal jugular, supraclavicular lymphatics	Para-aortic lymphatics
Infraclavicular, axillary, and pectoral lymphatics	Mesenteric lymphatics
Epitrochlear and brachial lymphatics	Iliac lymphatics
Mediastinal lymphatics	Inguinal and femoral lymphatics
Hilar lymphatics	Popliteal lymphatics

can be divided into those with limited disease (stage I and II) and those with advanced disease (stage III and IV). Patients with stage II and extensive ("bulky") disease are classified as localized or advanced disease based on additional risk factors.

Lymphonodal regions are divided into the following groups **(Table 2)**. (22)

THE MODERN TREATMENT OF HODGKIN LYMPHOMA

The modern treatment of Hodgkin lymphoma is multidisciplinary. The treatment begins with a biopsy of the affected lymph node and pathohistological diagnosis, followed by all available modern diagnostic methods to stage the disease. Based on the stage, the treatment is carried out using chemotherapy and radiation therapy. The first international study EuroNet PHL C1 was started in 2007 and it lasted until 2013, in which PET-CT first appeared as a mandatory diagnostic method for all therapy groups, based on which an indication for radiation therapy was established. This study included a population of children aged 0 to 17 years. The primary goal of this study was to maintain good disease control while reducing morbidity caused by the treatment.

Currently, the second international study EuroNet PHL C2 is underway, which includes patients aged 0 to 17 years who have histopathologically confirmed classical Hodgkin lymphoma. The study protocol stratifies patients into three therapeutic groups. In all three therapeutic groups, in the event of an unfavorable response after the application of two cycles of chemotherapy according to the OEPA protocol, there is an indication for radiation therapy.

In all therapeutic groups, after the histopathological diagnosis of classical Hodgkin lymphoma and adequate staging, the treatment begins with two cycles of chemotherapy according to the OEPA protocol, consisting of vincristine, etoposide, prednisone, and doxorubicin. After the second cycle of chemotherapy, according to the protocol, it is mandatory to perform a PET-CT, and based on the findings, an indication for radiation therapy is established. In case of an adequate response – complete remission (CR) confirmed by PET-CT, or partial morphological remission (PR) with a negative PET-CT– radiation therapy is not indicated. In case of an inadequate response, which implies the absence of complete or partial remission of the disease (without CR or PR), and with a positive finding on PET-CT, radiation therapy is indicated. In case of an indication for radiation therapy, it is necessary to irradiate all initial sites of the disease based on the PET-CT made at the time of the diagnosed disease using the "involved site" technique, and in case of PET-CT positive regions after completing chemotherapy in the second and third therapy group, it is indicated to conduct a radiotherapy "boost" on the area of PET-CT positive regions. After radiation therapy, depending on the stage of the disease and the response to the applied treatment, chemotherapy could be given according to this study protocol. Following the international recommendations, pediatric radiotherapy should be performed in radiation therapy centers with experience in pediatric radiation oncology. (25)

In case of relapsed/refractory pediatric Hodgkin lymphoma it is possible to utilize novel immunotherapy with chemotherapy regiments. For example, combination of brentuximab vedotin (CD30 antibody with conjugated mitostatic) and bendamustin for relapsed or refractory disease show promising results with 3-year-event free survival (EFS) and overall survival (OS) of 65% (26).

New treatment strategies incorporate immunotherapy agents like brentuximab vedotin in frontline treatment of high risk pediatric Hodgkin lymphoma patients. For example, a branch of protocol is developed where brentuximab vedotin replaces each vincristine in OEPA/COPDAC chemotherapy regimens. Radiotherapy is given as "involved node" radiotherapy only to PET-CT positive nodes after an incomplete PET-CT response upon two cycles of chemotherapy + immunotherapy. With this approach, 35% of patients achieved complete remission after two cycles of chemotherapy + immunotherapy and in these patients radiotherapy was omitted. Also, with this approach, the 3-year EFS was 97.4% and the OS was 98.7%. (27)

Introduction to radiotherapy of pediatric Hodgkin lymphoma

Until the 1960s, treatment outcomes for Hodgkin lymphoma were modest. However, the development of linear accelerators and the use of large radiation fields significantly changed the disease prognosis (28). In the past, radiotherapy involved total lymphatic irradiation, total nodal irradiation, and extended field radiotherapy. Total

lymphatic irradiation of the supradiaphragmatic region included lymphatics of the oropharynx and nasopharynx, the mantle field covering lymphatics of the neck, supra and infraclavicular regions, axilla, mediastinum, and abdominal lymphatics. Total nodal irradiation involved the supra- and infradiaphragmatic region, lymphatics of the oropharynx and epipharynx, the mantle field, and the inverted Y field covering para-aortic lymphatics, iliac lymphatics, inguinal lymphatics, and spleen. Extended field radiotherapy covered affected lymphatic regions as well as adjacent unaffected lymphatic regions. The second significant step in the treatment occurred in the 1960s with the introduction of chemotherapy using the MOPP protocol consisting of mechlorethamine, vincristine, procarbazine, and prednisone (28). With the introduction of adequate systemic therapy, radiation fields gradually decreased from total nodal irradiation, through extended and involved field radiotherapy, to modern involved site and involved node radiotherapy. Another important protocol is ABVD, consisting of doxorubicin, bleomycin, vincristine, and dacarbazine.

"Involved field"

"Involved field" radiotherapy with radiation fields includes positive disease sites with a safety margin of 1.5-2 cm in all directions. It was traditionally used during conventional (2D) radiotherapy. According to the European protocol HL Studien HD16, the radiotherapy fields for "involved field" radiotherapy are defined as follows (29) **(Figure 1)**:

- In case of positive cervical lymph node regions, the radiation field must include ipsilateral or contralateral cervical lymphatics, depending on the extent of the disease, caudally including supraclavicular lymphatics and 2/3 of the clavicle.
- In case of disease in the submental and submandibular lymph nodes of the neck, the radiation field should include ipsilateral submental, submandibular, cervical, and supraclavicular lymphatics.
- In case of supraclavicular localization of the disease, the field encompasses the ipsilateral supraclavicular region and 2/3 of the clavicle, cranial ipsilateral cervical lymphatics up to the hyoid bone, and if necessary, caudally including the infradiaphragmatic region.
- In case of axillary localization of the disease, the field extends cranially from the clavicle to the 5th or 6th rib and medially extends 1 cm into the lung parenchyma.
- If the disease is present in the upper mediastinum, above the tracheal carina, the field includes cervical lymphatics bilaterally up to the hyoid bone, medial 2/3 of the supraclavicular region bilaterally, and caudally extends one vertebra below the tracheal carina.
- In the case of involvement of the mediastinum below the tracheal carina, the radiation field extends cranially one vertebra above the trachea, and caudally extends to the diaphragm at the level of the 10th or 11th thoracic vertebra (Th10 or Th11). If the hilum is not affected, the field width includes the transverse processes of the vertebrae.
- If the disease is present in the middle mediastinum, the field extends from the jugulum to the diaphragm at the level of the 10th or 11th thoracic vertebra (Th10 or Th11). Positive hilar lymphatics are included with a lateral safety margin of 1.5 cm.
- If para-aortic lymphatics or lymphatics in the splenic hilum are positive, the field extends from the 10th or 11th thoracic vertebra (Th10 or Th11) to the lower edge of the 5th lumbar vertebra (L5), including the splenic hilum with a lateral safety margin of 1.5cm.
- In case of positive inguinal-femoral lymph nodes, they must be included in the radiation field.

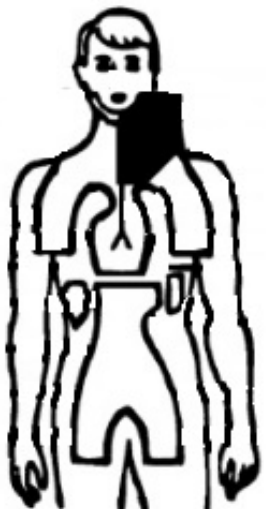

Figure 1. Example of "involved" field radiation fields. Taken from: https://en.ghsg.org/radiotherapy

With the advancement of chemotherapy and the development of 3D conformal radiotherapy (3DCRT), modified "involved" field radiation fields have been developed. These fields are based on target volumes with a safety margin of 1-2cm, which has allowed for significantly smaller volumes than 2D conventional "involved field" fields (23) **(Figure 2)**.

"Involved node" and "involved site" radiotherapy

In most cases (83%), the site of relapse in early stages of Hodgkin lymphoma is represented by the initially affected lymph nodes (30). It is also believed that modern chemotherapy regimens are sufficient in eliminating micrometastases in radiologically normal lymph nodes. Additionally, large radiation fields have a high frequency of late side effects, most commonly in the form of cardiovascular mortality and mortality caused by secondary neoplasms (28). "Involved node" and "involved site" radiation therapy volumes are based on the use of 3D CRT and are defined as follows:

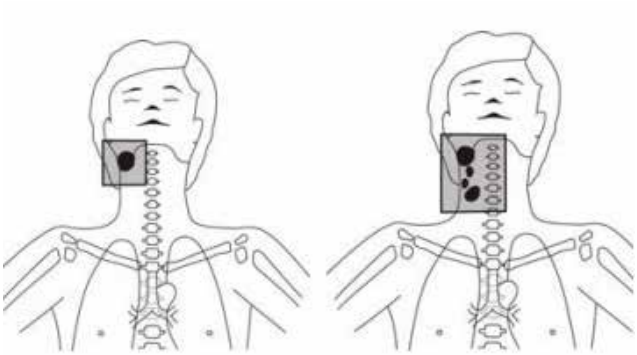

Figure 2. Example of modified 'involved field' radiation fields in case of right-sided cervical lymphadenopathy. Retrieved from EuroNet PHL C1 protocol.
Note: In case of 2D conventional radiotherapy, the 'involved field' would cover all lymph nodes on the right side of the neck, including the supraclavicular region.

- GTV (Gross Tumor Volume) is the volume visible on diagnostic methods and it usually represents a changed lymph node.
- CTV (Clinical Tumor Volume) represents the volume at risk of existing microscopic disease.
- PTV (Patient Tumor Volume) is defined by forming a safety margin around the CTV to compensate for inter- and intrafractional movements.

The "involved node" technique is based on forming a narrow margin around the affected lymph node, while excluding uninvolved lymphatics and surrounding tissues from the radiation volume (31). This technique requires the availability of PET-CT, which needs to be taken in the same position as CT for radiotherapy planning. In this way, the PET-CT and CT for radiotherapy planning can be co-registered (overlapping in the radiation therapy planning system), making it possible to include the changed lymphatic in the radiation field extremely precisely (32). According to the current Euronet PHL C2 protocol, after completed chemotherapy treatment, a PET-CT is necessary, where in case of a PET-positive lymph node larger than 10mm, an indication for radiation therapy is set. In this case, GTV represents a PET-positive lymph node, and the CTV margin is formed by expanding the GTV by 5mm. The PTV margin is defined by the technical characteristics of the radiotherapy center and in most centers it amounts to 5mm.

When it comes to "involved site" radiation therapy, it is based on the same principles as "involved node" but allows for wider margins in case of insufficiently precise diagnostic procedures. For example, if PET-CT was not taken in the same position as the CT for planning, the PET-positive lymphatics will not ideally match during co-registration (overlapping of diagnostic images of PET-CT and CT for planning), and a larger CTV margin is needed to ensure that the PET-positive lymph node is not outside the radiation volume. (24). The "involved field" CTV according to the PHL C2 protocol covers all initial sites of disease in the cranio-caudal direction with a 5mm margin, while the lateral margin is formed based on post-chemotherapy diagnostic findings with a 5mm margin, with the note that when defining CTV, it is necessary to take into account the reduction of disease volume after chemotherapy and the change in the relationship of anatomical structures (24). Techniques that enable the reduction of radiation volumes directly affect the reduction of late toxicity and improve the quality of life of treated patients.

The procedure of modern radiotherapy planning.

The first step in planning radiotherapy for pediatric Hodgkin lymphoma is CT for radiotherapy planning, which is performed in the supine position with arms slightly bent at the elbows and away from the body. Patient immobilization is performed by making a thermoplastic mask for the head, neck, and shoulders **(see Image 3)**.

Figure 3. Thermoplastics immobilization mask for head neck and shoulders. *(Material from the Institute of oncology and radiology of Serbia)*

The scope of scanning during therapeutic CT depends on the localization of the disease. For example, in case of disease localized in the mediastinum and neck, scanning is most commonly performed from the base of the skull to the diaphragm. Even in children, the use of intravenous contrast is recommended during therapeutic CT to clearly visualize the neck and mediastinal blood vessels from enlarged lymph nodes. In case of disease in the mediastinum, especially the upper mediastinum, the use of the deep inspiration breath technique (DIBH) is advised because it can better spare organs at risk such as the heart and lungs. It is believed that the use of DIBH can reduce the mean dose to the heart, coronary blood vessels, and lungs by 15-20%. (33) When it comes to the

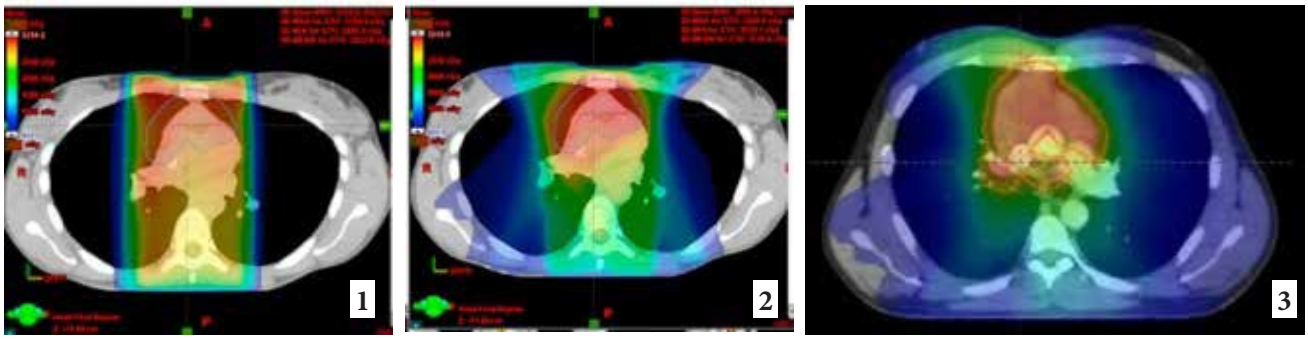

Figure 4. Comparation of CRT (1), IMRT (2) and VMAT dosimetry techniques (3). *(Material from the Institute of oncology and radiology of Serbia)*

pediatric population, the deep inspiration technique can only be used in children who are age-appropriate and cognitively capable of understanding and performing DIBH.

The modern radiotherapy techniques that are currently standardly used are: 3D conformal radiotherapy (3DCRT), intensity-modulated radiotherapy (IMRT), and volumetric arc therapy (VMAT). IMRT is characterized by the use of a small number of non-opposing fields to increase the conformality of the radiation volume and reduce the dose to organs at risk (OAR). The characteristic of VMAT technique is that it allows the highest degree of conformality, but large volumes of healthy tissue receive low doses of radiation ("low dose bath") (34), which theoretically may increase the chance of induced cancer. This technique is used with caution in radiotherapy of pediatric Hodgkin lymphoma (Figure 4). It must be taken into account that any radiotherapy carries a risk of developing secondary cancers, and IMRT also carries a risk of developing secondary cancers due to "scattered" radiation and the small volume of a child's body (34).

Proton therapy can also be used for the treatment of pediatric Hodgkin lymphoma. Namely, protons have the radiobiological property of releasing all their energy in the desired target volume and thus spare the surrounding healthy tissue and organs at risk (OAR) (35). Also, dosimetry studies have shown that the use of proton therapy reduces the mean dose to the OAR, for example to the heart and all cardiac structures, which is important for radiotherapy of Hodgkin lymphoma of the supradiaphragmatic region. (36).

Delineation is performed on the basis of modern protocols with PET-CT co-registration: initial, after several cycles of chemotherapy (interim) and after completed chemotherapy. In addition to the target volumes (GTV, CTV, PTV), OARs such as the heart, lungs, thyroid gland, spinal column are contoured when we talk about the supradiaphragmatic region **(Figure 5)**.

After the delineation of the target volumes and OAR, the radiotherapy plan is drawn up by a physicist and then the plan is analyzed – the coverage of the target volume with the prescribed dose and potential endangerment of the surrounding tissues and organs. **(Figure 6)**

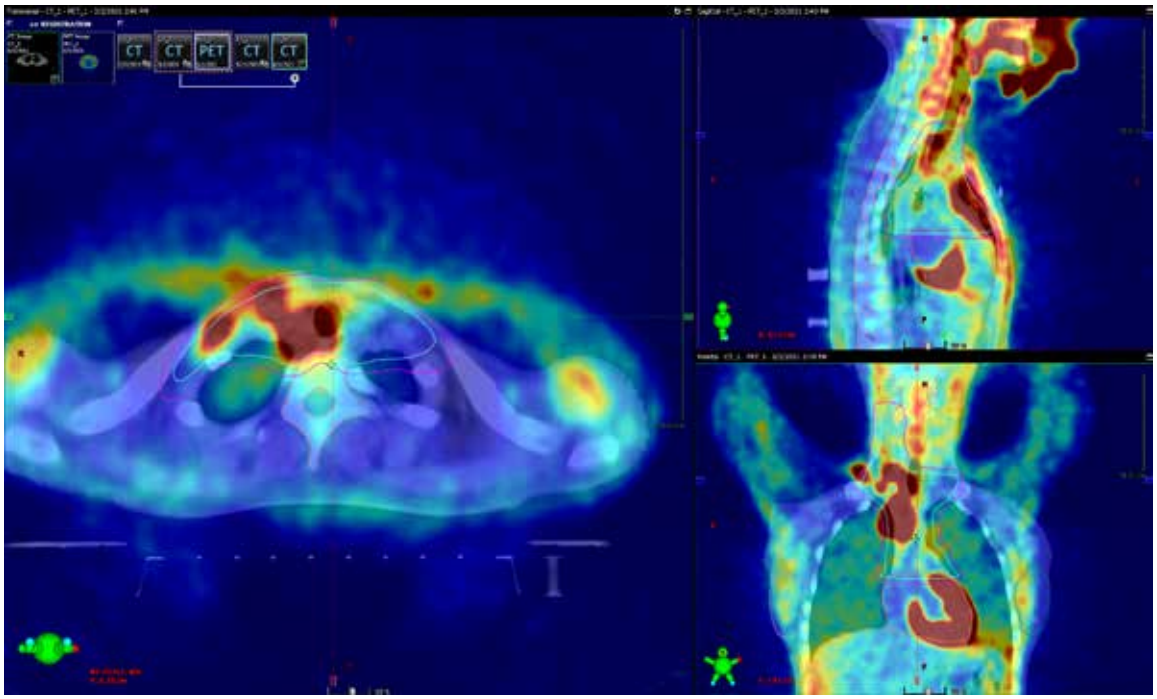

Figure 5. Delineation of Hodgkin lymphoma initially localized on the right side of the neck and anterior mediastinum using the 'involved site' technique with co-registration with the initial PET-CT. *(Material from the Institute of Oncology and Radiology of Serbia)*

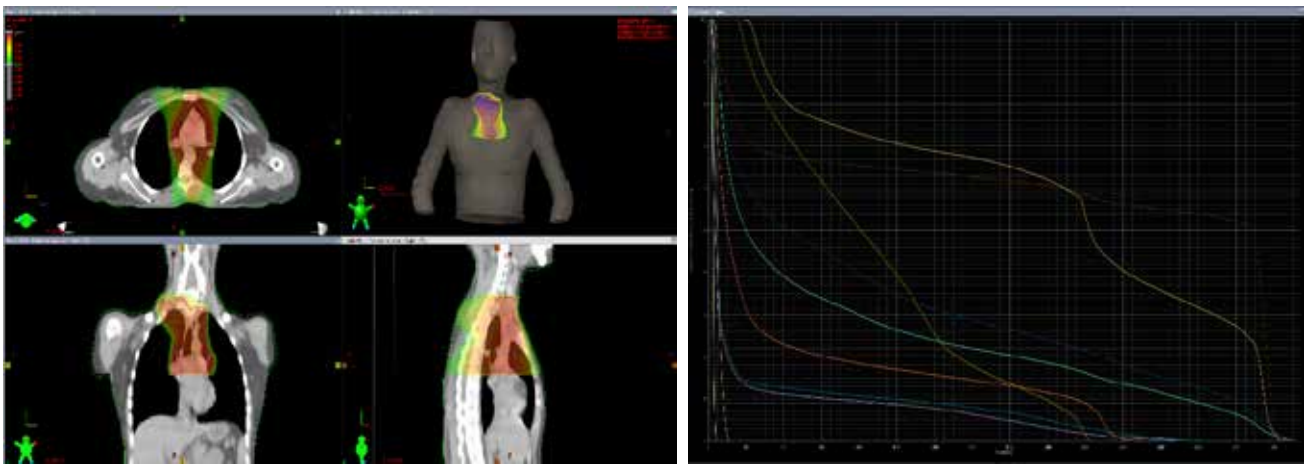

Figure 6. a) Coverage of the target volume with the prescribed dose. b) Dose-volume histogram which can estimate what volume of tissue receives a certain radiation dose. *(Material from the Institute of Oncology and Radiology of Serbia).*

ACUTE AND LATE TOXICITY OF RADIATION THERAPY

The immediate side effects of radiation therapy (RT) vary depending on the part of the body that is irradiated. In pediatric patients, lower doses are used, causing acute, reversible, unwanted toxicity such as changes in taste, dry mouth, inflammation of the esophagus, hair loss at the back of the head, redness of the skin, and sometimes bloating, nausea, and vomiting, depending on the organs and tissues that are within the radiation volume. Patients who undergo infradiaphragmatic radiotherapy may experience nausea and vomiting that is controlled by modern antiemetics. In radiation of large volumes, myelosuppression may occur, mainly manifested by mild leukoneutropenia, which can be more pronounced, especially when myelosuppression is potentiated by systemic therapy that is most often administered before radiation therapy.

Late toxicity of radiation therapy can manifest several years or decades after the radiation therapy. These are mostly musculoskeletal, cardiovascular, pulmonary, related to testicles and ovaries in pelvic radiation, with possibility of secondary malignancy occurrence. Late toxicity to the musculoskeletal system is usually manifested by lower growth compared to the age percentile. Lower growth with shortened clavicles and hypoplasia of the neck muscles particularly occurs when doses greater than 20 Gy are used in pre-pubertal age. (1) Late toxicity is the basis for reduced use of radiation therapy, as provided by new protocols such as EuroNet PHL C2.

Radiation therapy of the supradiaphragmatic region, particularly the "mantle field," is the cause of pulmonary pneumonitis and lung fibrosis, but the synergistic effect of bleomycin within the most commonly applied ABVD chemotherapy protocol must be taken into account. (37) Common late side effect of radiation therapy of the neck and upper mediastinum is also hypothyroidism. After 2.9 years after the completion of radiation therapy, 43% of patients develop biochemically manifest hypothyroidism. It is considered that a dose greater than 21Gy is sufficient to cause hypothyroidism. (38) In case of infradiaphragmatic disease, the ovaries and testicles may also be at risk. In female patients who were treated with radiochemotherapy at a younger age, there is a significant risk of premature menopause before the age of 20, the relative risk being 3.7. As for women treated in their youth, the risk of premature menopause also increases with age, so for women between the age of 21 and 25, the relative risk is 25%. It is expected that 43% of women who have turned 31 will enter menopause. (39) Regarding the male gender, a certain number of boys who were treated only with radiation therapy doses of 40 to 45Gy have managed to become fathers within 3-19 years after radiation therapy. (40)

People who have been treated with radiotherapy are at long-term risk of developing late toxicities, including radiation-induced malignancies (RIM). While Cahan et al. defined postradiation sarcoma in 1948, modified criteria are used nowadays to define RIM. According to these criteria, RIM must appear within treated volumes with a sufficiently long latent period and must have a different histology compared to primary malignancy, and the tissue origin of the RIM must be free of diagnosed metabolic and genetic malformations prior to radiation. (41) After multidisciplinary treatment of Hodgkin lymphoma, patients are most often at risk of developing leukemia (e.g., acute myeloblastic leukemia) as well as solid tumors (most often cancers of the thyroid gland, breast, and bone sarcomas). Leukemia, which occurs in the first 4 to 10 years after treatment, is primarily associated with alkylating agents as part of chemotherapy protocols (42). As for non-hematological malignancies, breast cancer occurs most often with a standardized incidence of 56.7, followed by thyroid cancer with a standardized incidence of 36.1, followed by bone, colorectal, lung and stomach cancer. (43)

Long-term follow-up of treated patients is of great importance, given that induced cancers occur even 30 years after the initial treatment of Hodgkin lymphoma. Regu-

lar and long-term follow up enable early diagnosis of secondary malignancies and adequate treatment.

CONCLUSION

Radiotherapy represents an important therapeutic approach in the combined treatment of pediatric Hodgkin lymphoma. Despite potential side effects, its application in combination with chemotherapy improved the survival rate of young patients. The introduction of modern radiotherapy techniques (3D CRT, IMRT and VMAT) and proton therapy enabled a more accurate coverage of the target volume and minimal damage to healthy tissue. Future research will be aimed at establishing a balance between the application of chemotherapy and radiotherapy with reduced side effects of combined treatment, which will enable preservation of the quality of life of cured patients.

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MOGUĆNOSTI RADIOTERAPIJE U LEČENJU PEDIJATRIJSKOG HOČKINOVOG LIMFOMA

Predrag Filipović[1], Jelena Bokun[1,2], Tatjana Arsenijević[1,2], Marina Nikitović[1,2]

Sažetak

Pedijatrijski Hočkinov limfom je maligno, limfoproliferativno oboljenje dece i adolescenata**.** Zračna terapija predstavlja važan vid lečenja. Mogućnost kasne toksičnosti radioterapije je ograničavajući faktor primene radioterapije kao modaliteta lečenja kod pedijatrijskih pacijenta.

Tehnološkim napretkom radioterapije, uvođenjem naprednih radioterapijskih tehnika i protonske terapije, poboljšana je preciznost zračne terapije i smanjen rizik od dugoročnih posledica. Ove tehnologije omogućavaju ciljano lečenje, značajno smanjujući izloženost zdravih tkiva i organa zračenju.

Sve postojeće preporuke lečenja i sprovedene kooperativne studije pokazale su da je zračna terapija efikasna u lečenju pedijatrijskog Hočkinovog limfoma, posebno kada se kombinuje sa hemioterapijom. Sa druge strane postoji mogućnosti kasne toksičnosti na organe u rastu i razvoju kao i nastanka sekundarnih maligniteta što mora biti pažljivo razmotreno prilikom odluke o sprovođenju radioterapije.

Zračna terapija predstavlja značajan terapijski pristup u kombinovanom lečenju pedijatrijskog Hočkinovog limfoma. Kombinovanim terapijskim pristupom poboljšani su rezultati lečenja, a napredne tehnike radioterapije smanjiće rizik za neželjene efekte. Indikacije za primenu radioterapije treba pažljivo proceniti u lečenju pedijatrijskih pacijenata sa Hočkinovim limfomom.

Ključne reči: pedijatrijski Hočkinov limfom, zračna terapija, kombinovano lečenje, toksičnost

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REVIEW

Pharmacological treatment of treatment-resistant depression: towards evidence-based recommendations

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Summary

Depression, a major global public health concern and leading cause of disability, necessitates effective management. This paper, as part of the development of comprehensive guidelines for the treatment of depressive disorder in Serbia, delves into the pharmacological treatment of treatment-resistant depression (TRD), focusing on augmentative and switching strategies, aiming to address the lack of response to standard treatments. The focus is on the efficacy and tolerability of various pharmacological agents, aimed at facilitating informed clinical decisions. In TRD, augmentation strategies involving atypical antipsychotics, NMDA antagonists, mood stabilizers, and other compounds are examined. Finally, the importance of an individualized approach in deciding between augmentation and switching strategies is emphasized. This narrative review aims to inform treatment guidelines and encourages a collaborative approach, which considers individual patient factors, to improve the quality of care for individuals with treatment-resistant depression.

Keywords: treatment-resistant depression, pharmacological treatment, guidelines

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INTRODUCTION

Depression, a leading cause of disability worldwide, presents a significant public health concern and has been the topic of recent investigations in Serbia (1). The effective management of this condition is paramount, and it requires a comprehensive understanding of the various treatment modalities available (2). This paper serves as a preparatory work for the development of comprehensive guidelines on the treatment of depressive disorders. These guidelines are being developed under the auspices of the Advisory Board on Mental Health of the Republic of Serbia.

Our aim is to provide a narrative review of the current literature on the pharmacological treatment of treatment-resistant depression (TRD). This work is intended to inform the development of evidence-based guidelines that will aid clinicians in making informed decisions about treatment options. This challenging condition, marked by a lack of response to standard treatments, necessitates a deeper understanding of alternative pharmacological strategies. We aim to provide a comprehensive narrative review of the current pharmacological options for managing TRD, drawing from the latest research and clinical guidelines. Brain stimulation strategies, in particular electro-convulsive therapy, has a strong evidence base as a treatment option for this condition, but lacks consistent guidelines (3)". However, it is important to note that our focus will remain on pharmacological interventions and will not include discussions on psychotherapeutic, brain stimulation, or other non-pharmacological interventions.

Our preparatory work is grounded in a thorough analysis of several authoritative sources in the field of psychiatry and mental health. These include the 14th edition of the Maudsley Prescribing Guidelines in Psychiatry (4), the American Psychiatric Association Practice Guidelines (APA) (5), the World Federation of Societies of Biological Psychiatry Guidelines (WFSBP) (6), and the Canadian Network for Mood and Anxiety Treatments Clinical Guidelines (CANMAT) (7). We also referred to the National Institute for Health and Care Excellence Guidelines (NICE) (8) for the treatment of depressive disorders in adults, as well as the recommendations of the Slovenian Medical Association (9). These comprehensive and well-respected resources have served as the foundation for the development of our recommendations, providing a robust basis for our analysis and subsequent guideline development. In addition, individual meta-analyses and RCTs were analyzed.

While we discuss various treatment strategies and their potential benefits and drawbacks, our aim is not to issue definitive recommendations. Instead, our goal is to inform and contribute to the ongoing dialogue on this topic. We encourage clinicians and policymakers to consider this information in conjunction with their professional judgment, patient preferences, and local regulations and practices when making treatment decisions. We also advocate for further research and guideline development by official health authorities to ensure the most effective and appropriate care for individuals with TRD. By providing a narrative review of the current evidence, we aim to facilitate the development of robust, evidence-based guidelines that will ultimately improve patient outcomes.

LACK OF THERAPEUTIC RESPONSE AND TREATMENT-RESISTANT DEPRESSION

Estimates suggest that 53% of patients with major depressive disorder, when treated with standard first-line psychopharmacological medications, fail to achieve remission. Moreover, 67% do not attain a satisfactory therapeutic outcome (10). Even with the implementation of a stepwise approach—where different antidepressants are sequentially employed—up to 20% of patients continue to experience significant symptoms beyond a two-year period (11).

Non-responsiveness to first-line pharmacotherapy is linked with numerous adverse outcomes. These include a decreased quality of life, an increased lifetime hospitalization rate, greater usage of emergency medical services, a higher risk of unemployment, and diminished productivity at work, relative to those who respond favorably to treatment (12).

Given the aforementioned factors, the concept of TRD, also known as refractory depression or "difficult-to-treat depression", was introduced (12). Various models have been proposed to quantify the lack of therapeutic response, but a definitive consensus on the precise definition of TRD remains elusive in literature. The most widely accepted definition of TRD encompasses the failure to respond therapeutically to two consecutive, adequate treatments using different antidepressants during a single depressive episode. However, there remains contention about what characterizes an adequate treatment, specifically, no well-defined criterion exists for sufficiently long treatment durations or adequately high doses of the recommended antidepressants (11,13). Moreover, ongoing discussions continue to debate whether TRD refers to a lack of a therapeutic response to any two antidepressants, or whether it specifies the absence of a response to two antidepressants from different classes, such as SSRIs and NaSSAs (14).

Current evidence suggests that an early improvement, defined as a minimum 20% symptom reduction after 2-4 weeks of treatment, is linked with the emergence of a therapeutic response and remission after 6-12 weeks of treatment (15). Conversely, the absence of early improvement after 2-4 weeks tends to predict a later lack of therapeutic response or remission. However, the literature lacks reliable evidence supporting the benefits of an early substitution of the initially prescribed antidepressant

(14). Consequently, it is suggested that for patients who do not demonstrate early improvement after 2-4 weeks, the antidepressant dosage should be increased in line with the therapeutic range and the patient's tolerance and the occurrence of side effects (7).

Pseudo-resistant depression

A considerable number of patients who fail to exhibit a therapeutic response may have "pseudo-resistant" depression, which does not equate to TRD. Pseudo-resistance primarily implies an inaccurate diagnosis of major depressive disorder. Often, these cases are instances of bipolar depression, where a lack of therapeutic response is anticipated given the divergent treatment approach compared to unipolar depressive disorder. Differentiating between bipolar and unipolar depression is sometimes very difficult in absence of indubitable signs of hypomania (16). In such cases, retracing of the steps to the history taking along with using particular approaches such as phenomenologically based interviews and assessments is recommended (17–19)

Pseudo-resistant cases also include instances where suboptimal antidepressant therapy doses have been prescribed, where the duration of the therapy was insufficient, or where treatment was discontinued due to poor tolerance of side effects, inadequate compliance, or any other reason (11,20). Notably, even when dosed according to guidelines, antidepressant treatment may not achieve therapeutic concentrations in the blood. Some patients are considered rapid metabolizers, which can lead to psychotropic active ingredients being eliminated and achieving lower blood levels than typical for the general population due to genotypic differences associated with the cytochrome P450 system (21). Additionally, from a pharmacokinetic perspective, careful documentation of a patient's concurrent, non-psychiatric therapy is essential due to potential drug-drug interactions.

While a diagnosis of major depressive disorder usually excludes organic causes in its etiology, a lack of therapeutic response demands a re-evaluation of the diagnosis. This is particularly important in the light of physical conditions stemming from endocrine origins (such as hypothyroidism or Cushing's syndrome), neurological conditions (of both cortical and subcortical origins), neoplastic diseases (like pancreatic cancer), autoimmune disorders (including systemic lupus erythematosus and overlap syndrome), vitamin deficiencies, and specific viral infections (22).

PHARMACOLOGICAL APPROACHES TO TREATMENT-RESISTANT DEPRESSION

Pharmacological approaches to TRD encompass: (1) switching or optimizing antidepressant dose, (2) augmentation strategies.

(1) Switching or optimizing antidepressant dose

Evidence suggests that changing antidepressants in individuals lacking a therapeutic response constitutes a viable therapeutic strategy. Although some opinions suggest the subsequent antidepressant should have a different mechanism of action, several randomized controlled trials and meta-analyses have found no significant differences with regards to improvement in efficacy when changing an antidepressant within a group (e.g., replacing one SSRI with another), compared to an antidepressant from another group (i.e., with a different mechanism of action, e.g., replacing an SSRI with bupropion) (23). On the other hand, one meta-analysis indicated a benefit when substituting an SSRI antidepressant with another class antidepressant (bupropion, mirtazapine, and venlafaxine), potentially providing an efficiency gain in the therapeutic response (28% non-SSRI versus 23.5% SSRI). Despite the current lack of consensus on this matter in literature, it is essential to note that individual differences in efficacy within SSRI antidepressants, albeit small, do exist (24). In line with this, after non-response, using a more effective medication when switching is advised.

(2) Augmentation

Augmentation refers to the addition of a new medication to the existing antidepressant therapy that did not yield a satisfactory therapeutic response (11). The results of randomized controlled studies, as well as a variety of international guidelines suggest that effective augmentation strategies can include atypical antipsychotics, NMDA antagonists, mood stabilizers, antidepressants, and other compounds.

Atypical antipsychotics

Given that atypical antipsychotics are drugs that exert their effects through activity on a wide range of receptors, including serotonergic receptors, a large number of randomized controlled trials have been conducted in recent years using these drugs as augmentation therapy in cases of inadequate response to first-line antidepressants, most commonly SSRI/SNRI.

Aripiprazole. Among atypical antipsychotics, aripiprazole is one of the most studied in TRD. Its effectiveness in TRD is thought to be based on its activity as a partial antagonist for various serotonin receptors (5-HT1A, 5-HT2A, 5-HT2B, 5-HT6, and 5-HT7) (25). Even using a rigorous and commonly used clinical definition of TRD (absence of therapeutic response to two adequate doses of applied antidepressants for long enough), results from four randomized controlled studies have so far shown that aripiprazole represents an effective augmentation strategy (26). Furthermore, one randomized controlled study found that the use of aripiprazole at lower doses did not increase the level of side effects compared to placebo (27).

Quetiapine. Sharing structural similarities with clozapine, quetiapine, a second-generation antipsychotic, exhibits differing receptor activity properties based on dosage. At the 50 mg dosage level, quetiapine primarily serves as an H1 receptor antagonist, inducing notable sedative effects. When the dosage is increased to 300 mg, quetiapine inhibits the 5-HT2C receptor and the norepinephrine transporter (NET). This particular characteristic is believed to underpin its antidepressant capabilities. With even higher dosages (800 mg), quetiapine blocks over 60% of D2 receptors, providing it with an antipsychotic action (28). Quetiapine's effectiveness as an augmentation strategy in TRD has received validation from several randomized controlled trials (28). One of these studies demonstrated that quetiapine outperformed lithium, a treatment historically recommended as the first line of augmentation in TRD (29). Quetiapine was notably effective in alleviating anxiety symptoms in patients with depression (30). As a result, the utilization of quetiapine as an augmentation strategy for individuals with TRD is particularly advisable, especially when patients exhibit persistent anxiety and symptoms of insomnia (28).

Risperidone. In addition to antagonizing D2 receptors, risperidone is also an antagonist of 5-HT2 receptors. Studies in TRD have shown that risperidone reduces depressive symptoms compared to placebo. However, despite the considerable effect sizes, risperidone has not undergone the same level of scrutiny in randomized controlled trials as compared to quetiapine and aripiprazole. Moreover, it is believed to be less well-tolerated than these aforementioned medications, particularly due to the more common incidence of hyperprolactinemia (28).

Olanzapine. Olanzapine is an atypical antipsychotic that has greater activity on 5-HT2A receptors than on D2 receptors in terms of antagonism. Additionally, olanzapine is an antagonist of 5-HT2C receptors, which is why it is thought to affect affective symptoms in some patients (28). To date, two randomized controlled studies have examined and demonstrated the efficacy of olanzapine as an augmentation strategy in cases of therapeutic non-response in depressive disorder. Compared to aripiprazole, quetiapine, and risperidone, the efficacy of olanzapine as an augmentation strategy in TRD is the lowest, according to the current evidence" (26). Also, as with risperidone, poor tolerance of olanzapine has been demonstrated, in terms of a higher burden of side effects, primarily in the form of metabolic disorders (31). On the other hand, it has been shown that the specific combination of olanzapine/fluoxetine has significant efficacy in TRD and may potentially bring more benefits than augmenting other SSRI/SNRI psychotropics with olanzapine (32).

Brexpiprazole. A third-generation antipsychotic, the most recently developed, brexpiprazole, achieves its effect through partial agonism of D2 receptors. Several studies have shown its effectiveness compared to placebo in patients with a lack of therapeutic response (33,34). However, considering the relatively recent appearance of this drug, and the smaller number of meta-analyses that included this drug in the analysis (26,35), its advantage over existing antipsychotics as augmentation agents has not been fully clarified yet. There is some evidence for a variety of other atypical antipsychotics in depression, such as cariprazine, but much of it is based on case reports (36), or either low quality or insufficiently rigorous research.

NMDA antagonists

Recent studies of new psychopharmaceutical agents have begun investigating drugs that function through full or partial antagonism of NMDA receptors, which are found on GABA-ergic interneurons that modulate glutamatergic transmission. The antagonism of NMDA receptors is hypothesized to activate various signaling pathways, leading to increased local protein synthesis, which in turn enhances the surface area of dendritic spines and improves the impaired synaptic activity of neurons in depression (37). This category of drugs includes D-cycloserine, minocycline, and ketamine. Given their experimental status and a lack of widespread use in clinical practice, D-cycloserine and minocycline are not evaluated in this paper.

Ketamine. Several meta-analytic studies examining TRD augmentation strategies have found ketamine to be effective (26,35). A recent meta-analysis indirectly comparing TRD augmentation agents, including atypical antipsychotics, mood stabilizers, and NMDA antagonists, suggests that the most likely positive outcome is achieved by augmenting the initial antidepressant with NMDA antagonists (35). Using the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) framework, it was shown that NMDA antagonists as augmentation agents for TRD currently hold a high level of evidence (35). However, given the relatively short period of ketamine's clinical use, its side effects may be underestimated, and its tolerability overestimated, particularly during repeated administration (38). It is important to note that there are different formulations of ketamine. Intravenous infusion of ketamine (0.5 mg/kg over 40 minutes) is considered the gold standard, but the efficacy of intranasal spray has been confirmed in multiple randomized controlled studies (39,40). Recently, both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved esketamine nasal spray for TRD in adults (41). Compared to other augmenting agents like atypical antipsychotics or mood stabilizers, esketamine offers a different mechanism of action, as well as rapid onset effects, and may be particularly useful in cases where these traditional augmenting agents have failed or are not suitable (42). Nevertheless, the clinical decision to use esketamine must weigh its rapid onset of action against factors like its side effect profile, the need for clinical supervision during adminis-

tration, and concerns about long-term safety and potential for abuse (43). Moreover, the cost and accessibility of esketamine may also influence its use in clinical practice, particularly when compared to other more established and possibly less expensive augmenting agents (44).

Mood stabilizers

Lithium. Efficacy of lithium as an augmenting agent for major depressive disorder was established in older studies, often in combination with Tricyclic Antidepressants (TCAs) (11,45). Randomized controlled trials that implemented lithium augmentation of SSRI demonstrated this strategy's effectiveness, albeit with a wide confidence interval (7,46). Recent meta-analyses, adhering to the conventional clinical definition of TRD, indicate that lithium continues to be a reliably effective augmentation strategy for these patients (26,35). When augmenting antidepressant therapy with lithium, it's crucial to establish a dosing regimen achieving blood levels of at least 0.4 mmol/l (47).

Lamotrigine. Meta-analytical study findings indicate that lamotrigine, as a SSRI therapy augmenter, is effective and well-tolerated (48,49). The negative aspects of employing lamotrigine as an augmentation strategy include the necessity for slow titration and uncertainties regarding the dosing regimen (4).

Even though there have been some investigations into the use of carbamazepine and valproate as augmenting agents for TRD, rigorous research yielding high-quality evidence supporting their efficacy remains notably scarce. Notably, some data indicate that valproate produces antidepressant-like effects in animal models (50). Also, while some open-label (51) and pilot studies (52) demonstrated efficacy in TRD, the availability of robust data, to the best of our knowledge, remains insufficient. In the case of carbamazepine, one study showed no gain in efficacy after augmentation of mirtazapine (53). In line with this, authoritative resources such as Maudsley Prescriber Guidelines 14th edition, as well as most recent guidelines on approaches to TRD do not endorse the use valproate or carbamazepine in TRD (4,9).

Antidepressants

Mirtazapine. Mirtazapine is a well-established antidepressant that has proven effective as a first-line treatment for depressive disorders when used in monotherapy. Two meta-analytic studies that included a significant number of randomized controlled trials examining the use of mirtazapine as an augmentation agent for TRD suggested that this drug might be effective when used in combination with SSRI and SNRI therapy (54,55). However, a recent high-quality randomized controlled trial did not demonstrate the efficacy of mirtazapine for this particular indication (56).

Bupropion. The results of the STAR*D study showed that bupropion was an effective augmentation strategy for those who did not respond to the administration of citalopram (57). Its efficacy in TRD was later confirmed through randomized controlled trials and one meta-analysis (54). A particular advantage of augmenting SSRI/SNRI therapy with bupropion is the potential to reduce sexual side effects, which are very common during monotherapy with SSRIs/SNRIs (4). The summary of augmentation strategies in TRD is presented in **Table 1**.

Table 1. Summary of pharmaceuticals with significant evidence base effective for augmentation of treatment-resistant depression.

Augmenting agent	Dose range
Aripiprazole	2.5 – 15 mg
Quetiapine	150 – 300 mg
Risperidone	1 – 3 mg
Olanzapine	2.5 – 10 mg
Brexipiprazole	1 – 3 mg
Esketamine (intranasal)	28 – 84 mg
Ketamine (intravenous)	0.5 mg/ kg over 40 min.
Lithium	600 – 800 mg; 0.4 – 0.8 mmol/l serum level
Lamotrigine	100 – 200 mg
Mirtazapine	30 – 60 mg
Bupropion	150 – 300 mg

*Dosage ranges are presented based on evidence from randomized controlled trials and authoritative international guidelines referenced throughout the text

Augmentation or antidepressant switch?

As there are no clear indicators of using either augmentation or switching as a better strategy, several factors can influence this decision (7). Most importantly, the final decision should be tailored to each individual patient's characteristics. In general, it is recommended to consider switching antidepressant medication in the following cases: (a) when there is a lack of therapeutic response (less than 25% improvement) to the initial antidepressant; (b) when the patient experiences poor tolerability due to adverse effects of the first antidepressant; (c) when it is feasible to wait for a longer period for a therapeutic response (in cases of less functional impairment); and (d) when the patient expresses a preference to switch to a different antidepressant. On the other hand, augmentation is recommended in the following situations: (a) when there is an inadequate therapeutic response to two or more antidepressants; (b) when the first antidepressant is well-tolerated in terms of side effects; (c) when there is a partial therapeutic response to the first antidepressant (more than 25% but less than 50% improvement); (d) when residual symptoms persist after treatment with the first antidepressant, or when specific adverse effects can be targeted with augmentation agents; (e) when waiting for a therapeutic response is not feasible due to significant functional impairment; and (f) when the patient prefers adding psychopharmacological agents to the existing antidepressant treatment.

Polypharmacy should be approached with caution due to its potential to increase the risk of adverse effects and reduce treatment tolerability. While the analysis of literature provides valuable insights into the efficacy and tolerability of specific augmentation strategies, it is important to acknowledge that the individualized treatment plan for each patient should be developed through collaborative decision-making. It is crucial to consider individual patient factors that contribute to the therapeutic approach, as the therapeutic approach cannot be rigidly standardized.

CONCLUSION

In summary, we present a detailed narrative review of pharmacological approaches to TRD, with the goal of contributing to evidence-based clinical guidelines in Serbia. Our comprehensive analysis spans various therapeutic strategies for TRD, underlining the importance of tailoring treatments to individual patient profiles, which includes giving special attention to factors like the severity of depression and any coexisting conditions. Although our focus is on pharmacological interventions, we recognize the role of non-pharmacological methods, such as psychotherapy and brain stimulation, which are not covered in this narrative review. It is essential for clinicians and policymakers to integrate this knowledge with their professional expertise, taking into account patient preferences and the specific healthcare context of Serbia.

Author contributions

SJ and MI conceived of the work. SJ drafted the manuscript, synthetized the literature and critically revised the work. MI provided expertise in the interpretation of the data and critically revised the work. Both authors approved the final manuscript.

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FARMAKOLOŠKI TRETMAN TERAPO-REZISTENTNE DEPRESIJE: KA PREPORUKAMA ZASNOVANIM NA DOKAZIMA

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Sažetak

Depresija je jedan od vodećih uzroka invaliditeta i značajan javno-zdravstveni izazov, usled čega zahteva efikasan tretman. Ovaj rad predstavlja pregled farmakološkog tretmana i deo je izrade sveobuhvatnih smernica za lečenje depresivnog poremećaja. U ovom radu izložene su strategije za lečenje terapo-rezistentne depresije (TRD) koju karakteriše odsustvo odgovora na uobičajen tretman. Naglasak je na efikasnosti i podnošljivosti različitih antidepresiva, sa ciljem olakšavanja donošenja kliničkih odluka na osnovu dosadašnjih dokaza iz literature. Lečenje TRD može podrazumevati strategije augmentacije, ili zamene antidepresiva. U domenu strategija augmentacije, razmotrena je primena atipičnih antipsihotika, NMDA antagonista, stabilizatora raspoloženja i drugih biološki aktivnih jedinjenja. Odluka o izboru između različitih strategija augmentacije ili zamena antidepresiva temelji se na individualnim faktorima pacijenta. Ovaj sveobuhvatni pregledni rad teži da doprinese formiranju smernica za lečenje TRD, promovišući pristup saradnje i zajedničkog donošenja odluka između pacijenta i kliničara, uzimajući u obzir individualne specifičnosti pacijenta, radi poboljšanja nege osoba sa TRD.

Ključne reči: terapo-rezistentna depresija, farmakološki tretman, smernice

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REVIEW

Structural basis of increased bone fragility in aged individuals: multi-scale perspective

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Summary

Numerous epidemiological studies have shown that increased bone fragility and a higher risk of fractures are present in the aged, which reduces their quality of life and represents a significant socio-economic burden for the healthcare system. However, morphological and structural determinants underlying increased bone fragility have yet to be fully explained. This paper aimed to provide an overview of modern studies that dealt with determinants of increased bone fragility, analyzing different hierarchical levels of bone tissue organization (macro-, micro-, and nano-levels) in aged individuals and individuals with chronic comorbidities (mainly in individuals with chronic liver disease, renal disorders, and type 2 diabetes mellitus). Also, variable frequency of fractures at different skeletal sites in aged persons and individuals with chronic diseases was shown, indicating that aging-related bone loss is not a uniform process. A complete understanding of the spatial pattern of impaired bone quality can aid in the targeted evaluation of individualized fracture risk. Establishing a firm connection between the results of the clinical assessment of bone status and the analysis of numerous structural and mechanical bone properties (on various hierarchical levels) can represent a solid base for developing adequate guidelines and algorithms for prevention and treatment of increased bone fragility in aged individuals and individuals with chronic diseases.

Keywords: bone fragility, ageing, bone fracture, hierarchical bone organization, bone strength

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INTRODUCTION

Bone fractures are a significant public health concern that affects a considerable proportion of the global population, mainly aged individuals, but also individuals with various chronic comorbidities (1,2). Aging-related bone fragility can result in serious health consequences, leading to disability, reduced quality of life, and increased mortality (3,4). In addition, aging-related bone fragility has significant socioeconomic consequences (5). As a population ages, the number of individuals at risk for fractures increases, which puts greater demands on healthcare resources due to an increased need for hospital admissions, rehabilitation, and long-term care (6–8). Given that bone fractures are preventable, it is essential to fully understand how they occur, and which factors contribute to increased bone fragility in aged individuals and individuals with chronic comorbidities. By addressing the issue of aging-related bone fragility, we can improve individuals' health and well-being, reduce healthcare costs, and promote healthy aging.

Bone fractures in aged individuals most frequently appear at the femoral neck, radius, and vertebral column, with a predilection to affect postmenopausal women (9–11). Moreover, bone fractures in these individuals commonly occur due to low-energy trauma (predominantly due to a fall from a standing height) (12,13). If we want to fully understand the reasons for increased bone fragility in aged individuals (especially those aged 65 years and over) (14) and individuals with chronic diseases, we should consider two main factors: 1) the mechanical loads applied and 2) bone strength (resistance to fracture) (15,16). It is known that a mechanical impact generated during low-energy trauma *per se* could not be sufficient to cause bone fracture (17). Hence, the leading cause behind increased bone fragility in aged individuals must originate from characteristics of the bone itself. So, we must reject the common perception of bone as a simple unviable mineral connective tissue that provides structural support, protects internal organs, and facilitates movement, and put our best efforts into understanding bone as metabolically active and dynamic tissue.

STRUCTURAL MORPHOLOGY OF BONE TISSUE: SHORT OVERVIEW

Since bone tissue is a living and dynamic system made of complex nanocomposite material, it has different structural organization and morphology at different length scales **(Figure 1)**, allowing it to withstand mechanical

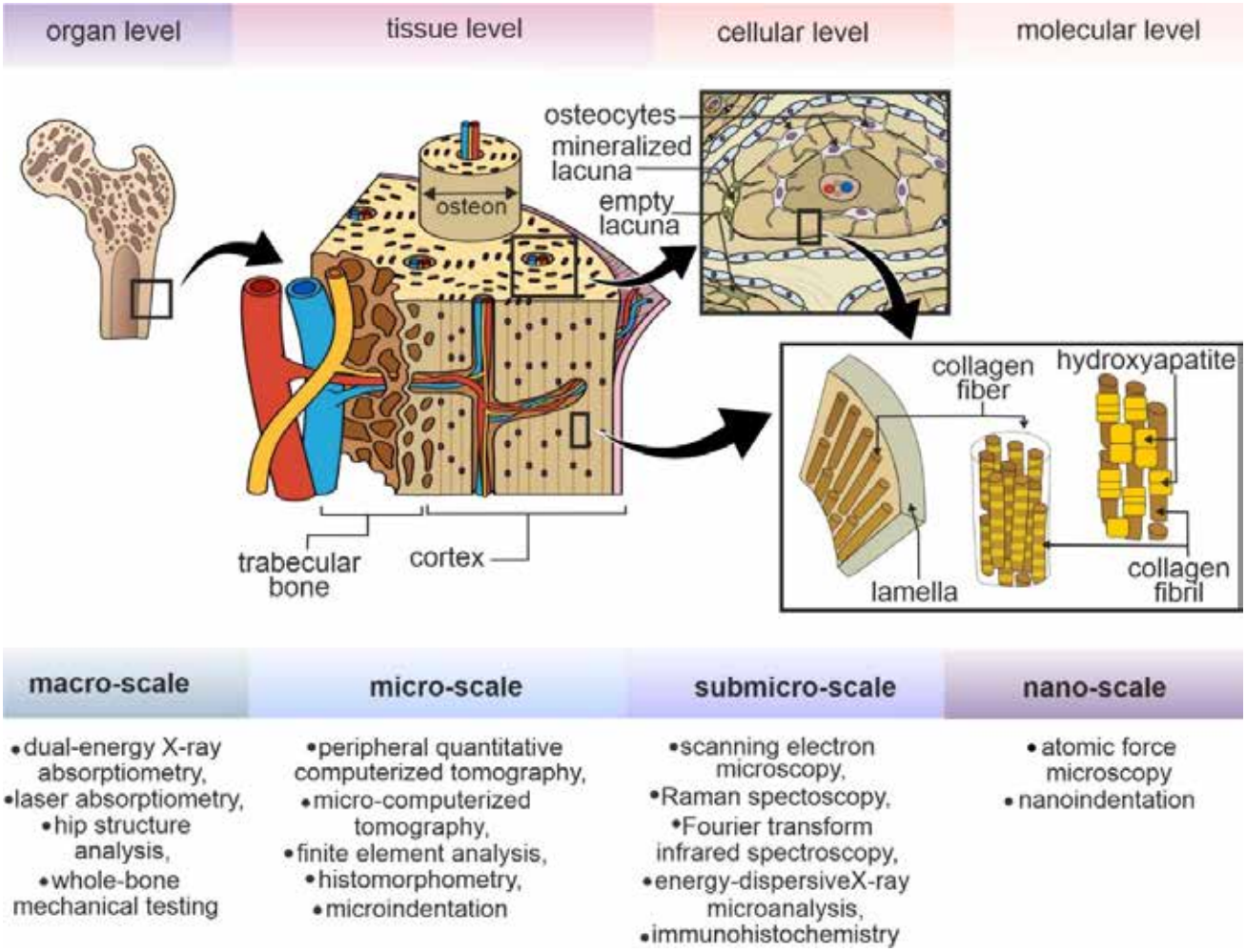

Figure 1. A schematic representation of the bone tissue hierarchical organization and methodology used for multi-scale bone assessment

loads while maintaining its structural integrity (18,19). Firstly, macroscopic observation shows the bone shape, size, and geometry (Figure 1), while cross-sectional analysis allows a distinction between two bone compartments: cortex (outer layer of bone tissue with a low porosity) and cancellous bone (porous bone tissue consisting of a network of interconnected bone trabeculae) (20). Microscopic evaluation of bone tissue reveals basic morpho-structural units predominantly found in cortical bone, known as osteons (21,22). Most of the bone volume is occupied by the bone matrix, inhabited by cells with specific functions: bone-forming cells called osteoblasts, bone-resorbing cells called osteoclasts, and the most numerous cells that act as bone remodeling orchestrators known as osteocytes (18,23). Going further to the submicroscopic level, it is evident that osteons consist of several concentric rings known as lamellae, while one lamella is made of many collagen fibers (Figure 1). At the nano-level, it is evident that each collagen fiber comprises many collagen fibrils immersed in an inorganic mineral component – hydroxyapatite crystals (19,24). Mineralized crystals and collagen fibers are combined in a highly organized manner to ensure that resistance to mechanical load is beyond the sum of mechanical characteristics of individual bone constituents (25,26).

Since bone is subject to morphological changes during aging (27,28) and various chronic diseases (such as chronic liver diseases, renal disorders and type 2 diabetes mellitus) (29–31), it is essential to investigate which bone characteristics (and at what hierarchical level of organization) could contribute to aging-related and disease-related bone fragility. Even though modern science is witnessing a significant breakthrough in technical inventions of bone-assessing medical imaging **(Figure 1)** (32), entirely accurate, reliable, and clinically relevant methods to assess bone fragility in aged individuals and individuals with chronic comorbidities are yet to be invented.

CLINICAL ASSESSMENT OF FRACTURE RISK: ADVANTAGES AND LIMITATIONS

Although recent attempts have been made to create a roadmap for improving global musculoskeletal health (33), there are still many unresolved issues in clinical assessments of fracture risk. Namely, the "golden standard" in the clinical estimation of the fracture risk is areal bone mineral density (aBMD) obtained by dual-energy X-ray absorptiometry (DXA). It is defined as bone mineral content (BMC; g) per analyzed bone area. The peak of aBMD values is reached in late adolescence, after which it remains stable, and then bone mass starts to decline (7,18,34). Aging-related bone loss is gradual in men, while accelerated bone loss is pronounced in postmenopausal women (13,35) due to the negative net bone balance (a decline in bone formation could not compensate for bone resorption) driven by hormonal dysregulation (34). Also, increasing the outer diameter and thinning of the cortical bone (periosteal apposition and endosteal resorption occur in a sex-specific manner during aging), contributing to increased bone fragility in aged individuals (36–38).

It is clear that a single two-dimensional parameter, such as aBMD, cannot fully reflect the fracture risk since many studies have suggested that eliminating low aBMD in aged individuals would reduce the risk of fractures only modestly (13,39). Moreover, the bone mass of individuals who sustained a bone fracture and those who did not experience bone fracture overlap considerably, indicating that bone mass and aBMD are insufficient for individual fracture risk prediction. Also, it has been known that some pharmaceutical agents used to treat osteoporosis positively affect bone strength and decrease fracture risk without increasing aBMD (40,41), indicating the necessity of using other bone characteristics in individualized fracture risk assessment.

Several attempts have been made to overcome the limitations of using aBMD for fracture risk assessment. Among them, DXA-based hip structure analysis (HSA) of the proximal femora allowed for estimating specific biomechanical indices of femoral bone strength (42,43). Indeed, despite its limitations, the HSA showed better sensitivity to predict hip fracture than areal BMD measurements alone (44) and improved understanding of the changes in bone strength components in aging and chronic diseases (45,46). Realizing that bone internal architecture is essential for fracture risk assessment, another valuable clinical tool, known as trabecular bone score (TBS), was developed (47,48). This grey-level textural measurement indirectly estimates bone microarchitecture from DXA images of lumbar vertebral column. Recent clinical studies have confirmed the fracture-discriminating ability of TBS in a substantial number of postmenopausal women (47,48). However, a significant limitation of this methodology is that it could be applied to one skeletal site only (L1-L4 vertebrae)(49). Also, another clinical fracture risk assessment tool, known as Fracture Assessment Tool (FRAX) was developed to demonstrate the 10-year probability of a hip fracture and of a major osteoporotic fractures (50,51), but it fails to recognize the impact of other non-skeletal fracture risk determinants (balance disturbances, reduced vision, and altered motor coordination that can cause an increased risk of falling) (50,51).

In clinical settings, histomorphometric analysis of transiliac bone biopsy samples has been used to quantitatively evaluate the bone status and effects of certain anti-osteoporotic therapies of bone tissue collected from aged patients (52–54) and patients with chronic comorbidities (55,56). Besides histomorphometry being an invasive procedure that allows 2D micro-scale bone assessment **(Figure 1)**, concerns remain because the iliac crest is not representative of various skeletal sites, given that osteoporosis is not a uniform process throughout the

skeleton. In addition, a modern noninvasive 3D *in vivo* method for clinical bone assessment at the distal radius and tibia is high-resolution peripheral quantitative computed tomography (HR-pQCT) (57–59). The radius and the tibia undergo different mechanical loading patterns (60) and have different fracture risks concerning age and sex, however these fractures are not the most frequent and not the most severe ones either. Nevertheless, numerous studies used HR-pQCT to address cortical and trabecular properties of the tibia and the radius in aged individuals (9,18). Also, an increasing number of studies is using HR-pQCT to reveal altered cortical and trabecular micro-architectural properties in individuals with diabetes mellitus (61), chronic kidney disorders (62) and liver diseases (63). Even though HR-pQCT analyses could provide better predictive accuracy of fracture risk assessment beyond aBMD measurements, it is yet to develop full potential due to high associated cost, limited voxel size (82 μm), and inability to access the most relevant fracture sites (proximal femora and vertebral column) (18). Considering the shortcomings of current methodology, it is of great importance to develop a better diagnostic algorithm that would allow reliable clinical fracture risk assessment in aged population and individuals with various chronic comorbidities.

MULTI-SCALE BONE ANALYSIS: REVIEW OF CURRENT LITERATURE AND FUTURE RESEARCH DIRECTIONS

Various factors (skeletal and non-skeletal) contribute to changes in fracture risk in aged individuals and individuals with chronic comorbidities that cannot be detected through bone mineral density. Commonly described as non-skeletal factors associated with higher fracture risk in aged individuals are sarcopenia, higher risk of falls, poor vision, altered motor coordination, therapeutic side effects, and disease complications (50). Among skeletal factors, the most significant attention in modern research is paid to impaired "bone quality"— generally referring to intrinsic bone properties (beyond aBMD) that influence mechanical performance (15,16).

In previous years, the relative contribution of micro-scale bone quality features to bone strength has been examined extensively. Namely, the aging-related trabecular micro-architectural decline is presented as a loss of trabecular elements, declined trabecular connectivity coupled with trabecular thinning observed at the proximal femora, vertebral bodies, the radius and tibia of aged individuals (64–66). Along with trabecular micro-architectural bone loss **(Figure 2)**, increased cortical porosity

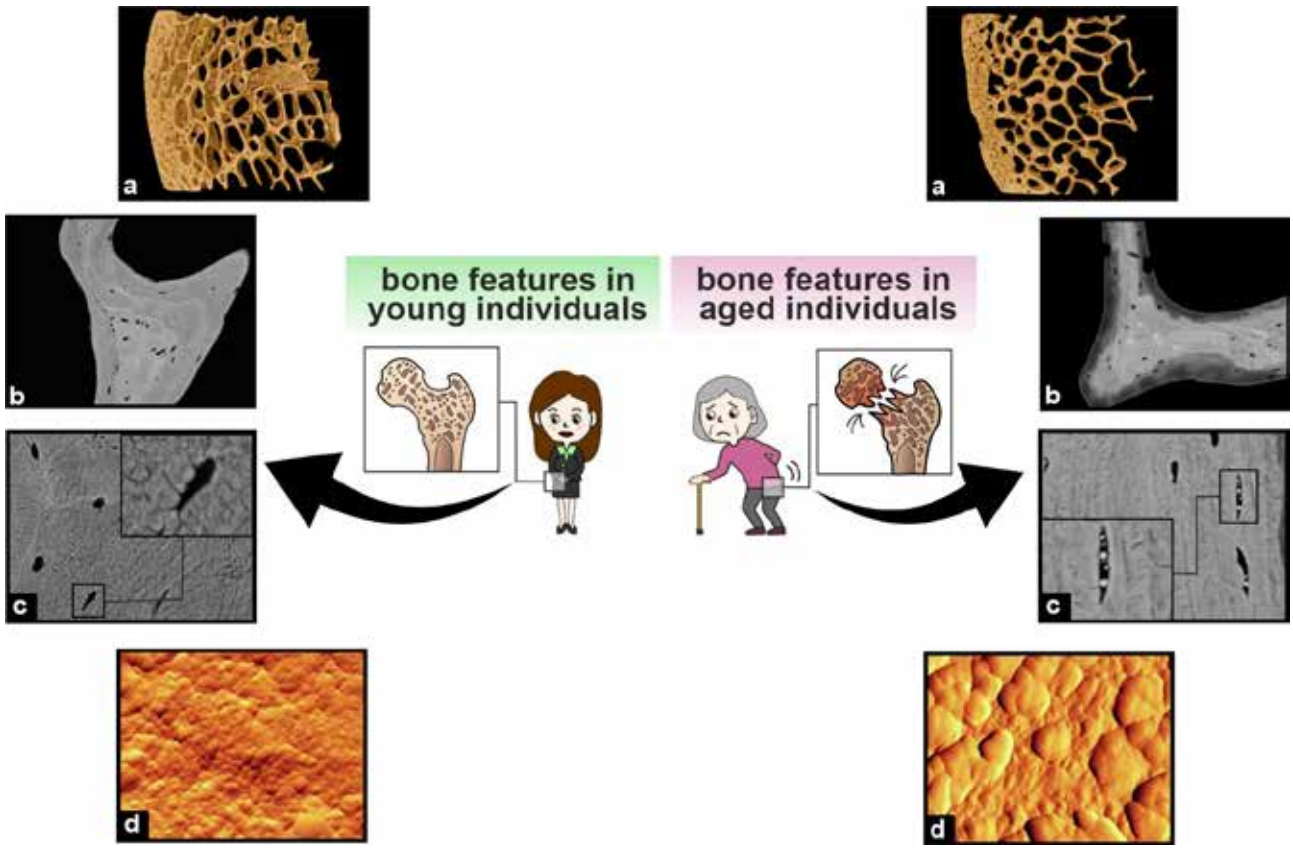

Figure 2. Small-length bone fragility determinants associated with ageing: graphic summary. Note significantly deteriorated bone micro-architecture (a) and altered bone tissue mineral content in aged individual (b). Moreover, osteocyte lacuno-canalicular network disruptions, increased number of mineralized osteocyte lacunas - micropetrosis (c), coupled with larger mineral crystal size (d) were demonstrated to contribute to aging-related bone strength reduction. However, the particular effects of various chronic diseases on these bone quality features are yet to be fully elucidated.

(originating from accumulation of incompletely closed osteons and resorption cavities) and cortical thinning are proven to contribute to bone fragility in aged individuals (11,64) and individuals with chronic liver diseases (29) and renal disorders (55). Moreover, it is becoming clear that spatial distribution of skeletal alterations and decline in intrinsic bone properties contribute to increased susceptibility to bone fracture in a site-specific manner (7,67). Namely, our team noted significantly different micro-architectural properties in the proximal femora, with different aging patterns between genders: the most prominent effect of aging in males was noted in the superolateral femoral neck (common fracture-initiating site), while the intertrochanteric region was most severely affected in females (65). These results support epidemiological data about the various occurrence of cervical and intertrochanteric fractures in older men and women (65). Also, having observed various levels of micro-architectural decline in the proximal femora, our team noted that the effect of chronic alcoholic liver diseases was not uniform, supporting epidemiological data about the association between chronic liver disease and heavy alcohol consumption and increased incidence of unstable intertrochanteric femoral fracture (46). Also, it was reported that duration, stage and severity of the disease could be an important risk factor for advanced bone alterations in individuals with chronic liver diseases (63,68). Lastly, it was revealed that vascular complications were important risk factor for femoral bone microstructural decline in individuals with type 2 diabetes mellitus (69), while hemodialysis was reported as a major risk factor for bone loss in patients with chronic kidney disease (70).

Conversely, the role of submicro- and nano-scale features is more difficult to study, especially *in vivo* or in clinical settings, pointing out that many questions related to these bone properties need to be explored to complete the bone fragility puzzle in aged individuals and individuals with chronic comorbidities. Recent studies revealed a shift to higher bone mineralization, reduced osteocyte lacunar density, and increased number of mineralized osteocyte lacunae (micropetrosis), coupled with deterioration in the lacuno-canalicular network which reduces the connectivity between osteocytes in the aged individuals (71–74). Moreover, a few state-of-the-art studies reported that increased mineral crystal size **(Figure 2)** could contribute to a decline in aging-related bone strength (24,27). On the other hand, micropetrosis was only recently investigated in individuals with chronic kidney disease (75), and its role in bone fragility of individuals with chronic liver diseases and type 2 diabetes is yet to be explored. Moreover, further research is needed to fully understand the contribution of each bone fragility determinant on various hierarchical levels of bone tissue (especially in relation to specific chronic disease), given that these data are a valuable resource that could (in integration with clinical data) make a solid base for generating specific algorithms for timely preventive and therapeutic measures for bone fragility related to aging and various chronic diseases.

CONCLUSION

Increased bone fragility is a common health problem in aged population and individuals with chronic diseases (especially chronic liver and kidney disease, and type 2 diabetes mellitus). Numerous studies have contributed to understanding the morpho-structural base of skeletal damage caused by aging and disease, but innumerable ambiguities remain. Thus, further research is necessary to solve the bone fragility puzzle in these individuals. Considering the need for patient-specific clinical guidelines for the prevention and treatment of compromised bone strength and its complications, the long-term benefit of multi-scale and advanced assessment of bone fragility could be in developing a specific diagnostic algorithm that will help to reliably predict bone strength based on the information available in the clinical context of each patient.

Author contribution statement

Conceptualization: all authors; Data acquisition: Jelena Jadzic; Data interpretation: both authors; Data visualization: Jelena Jadzic; Writing – original draft: Jelena Jadzic; Writing – Review and Editing: Marija Djuric; Project administration and Funding acquisition: Marija Djuric; Approval of the submitted manuscript version: both authors.

Ethical approval and patient consent

Not applicable.

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STRUKTURNE DETERMINANTE POVEĆANE KOŠTANE FRAGILNOSTI KOD STARIJIH OSOBA: VIŠESTRUKE PERSPEKTIVE

Jelena Jadzic [1], Marija Djuric [1]

Sažetak

Mnogobrojne epidemiološke studije su pokazale da je povećana fragilnost kosti i veći rizik od preloma prisutan kod starijih osoba, što redukuje kvalitet života i predstavlja značajan socio-ekonomski teret za zdravstveni sistem. Ipak, mofrološke i strukturne determinante koje leže u osnovi povećane koštane fragilnosti ovih osoba nisu u potpunosti razjašnjene. U ovom radu dat je pregled rezultata savremenih studija koje su se bavile determinantama povećane koštane fragilnosti analizirajući različite hijerarhijske nivoe organizacije koštanog tkiva (makro-, mikro- i nano-nivo) kod starijih osoba i osoba sa hroničnim oboljenjima (prevashodno sa hroničnim oboljenjima jetre, hroničnim bolestima bubrega i šećernom bolesti tipa 2). Takođe, pokazana je varijabilna učestalost preloma na različitim skeletnim mestima starijih osoba i osoba sa hroničnim oboljenjima, što ukazuje na to da gubitak kvaliteta kosti nije uniforman proces. Potpuno razumevanje prostornog obrasca narušenosti kvaliteta koštanog tkiva može pomoći u ciljanoj evaluaciji rizika od preloma kod svakog pojedinačnog pacijenta. Uspostavljanje veze između rezultata kliničke procene koštanog statusa i analize brojnih strukturnih i mehaničkih svojstava kosti (na različitim hijerarhijskim nivoima) može da predstvalja osnovu za razvoj adekvatnih vodiča i algoritama za prevenciju, dijagnozu i lečenje povećane koštane fragilnosti kod starijih i osoba sa hroničnim oboljenjima.

Ključne reči: fragilnost kosti, starenje, prelom kosti, hijerarhijska organizacija koštanog tkiva, čvrstina kosti

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REVIEW

Posttraumatic stress disorder – an overview in new diagnosis and treatment approaches

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Summary

Posttraumatic stress disorder (PTSD) is a complex condition, a common and disabling psychiatric disorder that causes immense suffering for millions of people. It is associated with a high rate of functional impairment, somatic complaints, a risk of suicide and comorbid psychiatric disorders, as well as extraordinary costs for health care system. The diagnosis of PTSD requires evidence of exposure to trauma, and is characterized by symptoms of re-experiencing, avoidance, and changes in arousal and reactivity. The American classification system added another cluster of symptoms related to negative changes in cognition and mood related to trauma, while the European classification system added complex PTSD as a new diagnosis. No evidence supported any particular intervention as a universal prevention strategy. CBT-TF, CBT and EMDR treatment had positive effects. Psychotherapy is the first line of choice in the treatment of PTSD. Trauma-focus interventions are recommended: CBT-TF, PE and EMDR, as well as stress management therapy. Pharmacotherapy approaches should start with one of the first-line options that include an SSRI such as fluoxetine, paroxetine, or sertraline, or the SNRI venlafaxine. Research evaluating combined psychological and pharmacological treatments for PTSD is limited and requires further study, but certain forms of PTSD require an integrative and multidisciplinary approach. Prevention, early detection, and clear treatment guidelines could be the best choice for every traumatized person as well as for the health care system.

Key words: posttraumatic stress disorder, traumatic experiences, diagnosis, pharmacotherapy, psychotherapy

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INTRODUCTION

The earliest records of humanity mention traumatic events and their consequences. In Gilgamesh (The Epic of Gilgamesh), the first great epic in the history of the human race, a vivid description of post-traumatic symptoms is given, suggesting that they are part of the fundamental human experience. Freud linked neurosis to trauma, describing the concept of traumatic neurosis in his early works (1). In the First and Second World War, disorders caused by stress, i.e., trauma, were described as "battle fatigue," "shock from shelling," "soldier's heart," and "survival guilt" (2). Contrary to literature, psychiatry has long had an ambivalent attitude towards the idea that reality can profoundly and permanently change human biology and psychology (3).

Post-traumatic stress disorder (PTSD) first appeared as a separate entity and was introduced as a diagnosable psychiatric disorder in the third edition of the Diagnostic and Statistical Manual of Mental Disorders - DSM-III (4) classification, following the knowledge of profound psychological effects of the Vietnam War (5) and concurrent studies on rape victims. It belonged to the group of anxiety disorders. In the International Classification of Disease (ICD-10) (6), it was introduced in the group of "Neurotic, stress-related, and somatoform disorders," in the category of "Reactions to severe stress and adjustment disorders." PTSD continues to attract debate, and it has experienced significant changes through the latest revisions.

Different kinds of traumas are unfortunately inevitable and frequently occurring in the contemporary world, with potentially devastating psychosocial consequences (7). Around the world, more than 50% of the general population experiences a traumatic event in their lifetime with the potential to develop PTSD, but most people experience multiple potentially traumatic events (8). About 3-7% of the adult population develops PTSD at least once in their lifetime (9). The lifetime prevalence of this disorder ranges between 1.9% (10) and 8.8% (11), but these rates are higher in post-conflict regions (12, 13). The incidence of PTSD after a traumatic experience is highly variable and can range from 0% to 100% (14). Therefore, not all individuals develop PTSD after facing trauma; a large number of people show a huge capacity and psychological resilience to recover after being exposed to trauma (15). Nevertheless, individuals also differ in flexible adaptation and effective coping with stress and trauma (16). A great number of potential risk and recovery factors are described, but determining why some individuals exposed to traumatic events develop PTSD and others do not is still a challenge (17). Comorbidity of PTSD and other mental disorders is almost a rule, but patients with PTSD have an increased risk of somatic disease (18, 19). Comorbid PTSD and depression are prevalent and are characterized by more psychological distress, with a high level of suicidality and poorer quality of life (20). PTSD is extraordinarily costly; it is one of the top psychiatric disorders that make sufferers use services of healthcare system, and its economic costs are among the highest (21).

DIAGNOSIS AND CLASSIFICATION

Diagnostic criteria for PTSD have some divergence between DSM-5 (22), ICD-10 (6) and ICD-11 (23), but for all three versions of classification systems, key inclusion criteria are exposure to a major traumatic event and characteristic symptoms including re-experiencing, avoidance, and an increased sense of threat. DSM-5 has broadened the definition of PTSD and classified it in the group of "Trauma and stressor-related disorders", which also contains Acute Stress Disorder (ASD), Adjustment disorder, Reactive attachment disorder (RAD) (applied only in children), Disinhibited social engagement disorder (DSED) (applied only in children), Other specified trauma and stressor-related disorder and Unspecified trauma and stressor-related disorder. Furthermore, DSM-5 does not divide into acute and chronic PTSD anymore and it incorporates a new symptom cluster of "altered cognitions and mood associated with a traumatic event". DSM-5 classification lists 20 symptoms, out of which there have to be at least six from four clusters (re-experiencing, avoidance, negative alterations in cognition and mood, and altered arousal) **(Table 1)**.

ICD-11 classification defines PTSD as primarily fear-based and classifies it in the group of "Disorders Specifically Associated with Stress" and its diagnosis requires three symptoms which include re-experiencing, avoidance and persistent perception of heightened threat (Table 1). Main differences between ICD-11 and ICD-10 include the fact that Acute Stress Reaction is not a mental disorder anymore and the fact that ICD-11 has included a new diagnosis, complex post-traumatic stress disorder (CPTSD). Complex PTSD is related to individuals who experienced one or more events that are extremely threatening or horrific in nature, which re difficult or impossible to escape from (24). The diagnosis of complex PTSD requires the presence of all three main symptoms of PTSD – re-expe-

Table 1. Comparison of diagnostic criteria DSM-5 and ICD-11 for post-traumatic stress disorder (PTSD)

DSM-5 criteria (APA, 2013)	*ICD-11 criteria (WHO, 2018)*
Intrusion symptoms	**Intrusion symptoms**
Avoidance	**Avoidance**
Negative alterations in cognition and mood	*Not applicable*
Alterations in arousal and reactivity	**Alterations in arousal and reactivity**
Not applicable	**Additional criteria for complex PTSD**

riencing, avoidance, sense of threat, as well as functional impairment. In addition, complex PTSD is characterized by severe and persistent problems in affect regulation, a negative self-concept which includes believing oneself to be diminished, defeated or worthless, and disturbed relationships, i.e., difficulties in sustaining relationships and in feeling close to others (25, 26). The type of trauma is a risk factor, not a requirement for the diagnosis of CPTSD. The need for a complex PTSD diagnosis has arisen from the existence of clinical presentations of symptomatology that extend beyond those described by the ICD-10 diagnosis of PTSD, particularly among individuals who experienced extreme, prolonged or multiple forms of trauma, and which have been reported by clinicians and researchers over several decades (27). CPTSD acknowledges pervasive symptoms that may result from experiencing chronic, prolonged traumatic events and finally, CPTSD is a distinct diagnosis and not a subtype of PTSD (23).

PREVENTION AND TREATMENT

A universal prevention strategy that would be applied to the entire population and used for individual prevention is under consideration. A variety of public health approaches to preventing traumatic events and providing psychoeducational messages through media to unselected populations both pre- and post-incident to anyone exposed to a particular traumatic event would be an example of a universal approach (28). Individual prevention strategies should be designed to prevent the onset of PTSD in individuals who do not meet ICD or DSM criteria but exhibit early symptoms. A various range of psychosocial approaches have been evaluated to prevent the development of PTSD. The latest evidence confirms and emphasizes the effectiveness of trauma-focused cognitive-behavioral therapy (CBT-TF), cognitive therapy and rapid eye movement desensitization and reprocessing therapy (EMDR), for the early treatment of people with PTSD symptoms in the first three months after the traumatic event (29-31). It has been concluded that it is more important to detect and treat people with significant symptoms than to prepare a package of general preventive measures (28). Debriefing trauma victims is strongly discouraged, both in individual and group sessions (30, 32, 33). The evidence of early pharmacological interventions in the prevention of PTSD is limited (34). Available data are inconsistent with the effectiveness of different pharmacological agents, including hydrocortisone, benzodiazepines, beta-blocker propranolol or selective serotonin reuptake inhibitor (SSRI) therapy (35-38). The evidence to support routine intervention after traumatic events involving many people (for example, terrorist attacks and natural disasters) is lacking. Consensus guidelines recommend supportive, practical, and pragmatic input but the avoidance of formal clinical interventions unless indicated.

Educational and organising preventive programs and interventions for traumatized people at the individual level, as well as at the collective level are necessary (39). It has been suggested that intervention right after the exposure to a traumatic event could be most efficient in preventing or attenuating a pathological response to stress-response disorders such as PTSD (16). Early interventions following traumatic events represent a heterogeneous group of approaches which include pharmacological interventions applied within a few hours of the traumatic event as well as early psychosocial assistance such as a single session psychological intervention for everybody involved within a month after traumatic events (40). Post-traumatic therapy is initially aimed at self-regulation and establishing control, the sense of security and predictability, and at taking engagement in an adaptive situation. Furthermore, it is aimed at controlling and managing physiological and biological stress reactions and finally at re-establishing secure social bonds and interpersonal effectiveness (41). The intervention focuses on improving coping skills, developing new coping strategies, considering changing the appraisal, improving personal resiliency, and obtaining social support from others, which can result in a reduction in negative consequences of stress such as PTSD (7).

Psychotherapy is the first line of choice in the treatment of PTSD, which has shown its unequivocal effectiveness, although some meta-analyses suggest that it is less effective than pharmacotherapy, especially in comorbidity with depression (42). According to the guidelines of the American Psychiatric Association (43), as well as the guidelines of the British Association for Psychopharmacology (44), pharmacotherapy and psychotherapy are the first line of choice. On the other hand, the National Institute for Clinical Excellence (30), the International Society for Traumatic Stress (29), as well as the Department of Defense for Veterans Affairs (45) claim that trauma-focus psychotherapy is more effective than pharmacotherapy.

Clinical guidelines based on evidence from systematic reviews and meta-analyses recommend trauma-focused psychological therapies (33, 46-48). Individual CBT with a focus on trauma, eye movement desensitization and reprocessing, as well as exposure therapy have been efficient **(Table 2)**.

Table 2. Psychotherapy approaches to posttraumatic stress disorder (PTSD)

Trauma-focused psychological therapies
Exposure therapy
Trauma focused cognitive therapy
Eye movement desensitization and reprocessing (EMDR)
Non-trauma-focused psychological therapies
Relaxation
Stress inoculation therapy
Interpersonal therapy

All trauma-focused psychotherapies have common components and common goals. They include imagined re-exposure to the event and exposure to real-life triggering cues typically avoided and all of them promote re-exposure to avoided memories, process emotional responses and correct cognitive distortions. Non-trauma-focus CBT – including relaxation training, stress inoculation therapy, self-regulation, interpersonal therapy, positive thinking – has been shown to have similar efficacy to trauma-focused CBT and EMDR immediately after treatment, but it does not remain so during follow-up (49). Non-trauma-focus CBT could be a valid substitution to trauma-focus therapy if trauma-focus is contraindicated or unavailable. Evidence suggests that phased approaches may be useful for more complex presentations of PTSD, but there is not enough research evidence (27). Stage-based approaches target specific problems such as dysregulation, dissociation, somatic symptoms to promote adaptive coping, a sense of safety and stabilization before undertaking any trauma-focused intervention (50).

Despite current guideline recommendations for first-line psychotherapy, pharmacotherapy is still often used as first-line treatment for PTSD. There is strong research evidence to support pharmacological treatment of post-traumatic stress disorder (PTSD) as a second line of trauma-focused psychological interventions. Results of meta-analyses as well as various guidelines recommend selective serotonin reuptake inhibitors (SSRIs) as a group and venlafaxine as first-line pharmacological treatments for PTSD (29,30,48,51,52) **(Table 3)**.

Table 3. Pharmacotherapy for posttraumatic stress disorder (PTSD)

First line
Fluoxetine, Paroxetine, Sertraline, Venlafaxine
Second line
Antipsychotics as a monotherapy (if antidepressant is not tolerated)
SSRI/SNRI + Antipsychotics (risperidone, olanzapine, quetiapine)
Mirtazapine – Augmentation for specific presentations, e.g., insomnia
SSRI/SNRI + Prazosin – The evidence is for augmentation versus monotherapy
Third line
Amitriptyline and Phenelzine
Mood stabilizers: carbamazepine, lamotrigine, gabapentin, topiramate, tiagabine, levetiracetam

There are still only 2 drugs approved by the US Food and Drug Administration for the treatment of PTSD: paroxetine and sertraline. The second line of pharmacological treatment of PTSD is represented by antipsychotics – risperidone, olanzapine, quetiapine (22, 33, 44, 52). Also, alpha-1 antagonist prazosin, as well as alpha-2A antagonist guanfacine, have been shown to be effective in the treatment of PTSD (53). Prazosin in combination with atypical antipsychotics has shown some efficacy in therapy-resistant patients with PTSD (54), while a growing number of recent studies show its efficacy in the treatment of nightmares (55, 56). Atypical antipsychotics as a second-line therapy can be given as monotherapy, if antidepressants are not tolerated, or as adjuvant therapy for refractory patients who have not had a favorable therapeutic response to SSRIs/SNRIs. These drugs are commonly prescribed to improve dissociation, hyperactivity, paranoia, psychosis, irritability, and aggression. The last step is to consider drugs from other classes with less evidence of effect, the occurrence of side effects, as well as insufficient clinical experience on their use. Thus, the third-line therapy could include amitriptyline or phenelzine, mood stabilizers such as carbamazepine, lamotrigine, gabapentin, topiramate, tiagabine, levetiracetam (48, 57). The increasing importance of the glutamatergic and GABA-ergic effects of anticonvulsants, as well as the discovery of abnormalities in these two systems among PTSD patients, has increased the level of interest in this class of drugs (58, 59). All anticonvulsants block sensitization and the so-called "kindling", although their mechanisms of action are different. From this class, medications that have shown their effectiveness are: carbamazepine, lamotrigine, gabapentin, topiramate, tiagabine, and levetiracetam (48). Benzodiazepines are strongly not recommended for the treatment of PTSD, as they showed not only ineffectiveness, but also worsening of symptoms in patients with PTSD (60).

There is some evidence that certain self-help programs, neurofeedback and yoga may be useful in alleviating PTSD symptoms (61-63). Acupuncture has been shown to be effective in patients who were waiting for CBT (64). Symptom-oriented hypnotherapy, transcendental meditation and breathing exercises have proven to be effective in a small number of cases (65). Australian guidelines support physical exercise in promoting general well-being (33).

CONCLUSION

Posttraumatic stress disorder (PTSD) is a complex condition, a multiclausal phenomenon that causes immense suffering for millions of people worldwide. This serious disorder does not strictly develop only in individuals affected by war or natural disasters; PTSD has been recognized as a much more pervasive problem that can affect literally any human being. The consequences could be devastating for individuals, as well as for healthcare systems, with significant costs. Understanding the nature of trauma, how and why the exposure to certain stressors causes psychological trauma, and how and in whom this leads to disabling disorder of PTSD, is an essential challenge. It is necessary to improve personal resilience, implement appropriate prevention, apply early diagnostics, as well as establish clear treatment guidelines with newer and novel therapeutic interventions.

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POSTTRAUMATSKI STRESNI POREMEĆAJ – PREGLED NOVINA U DIJAGNOSTIČKIM I TERAPIJSKIM PRISTUPIMA

Bojana Pejušković[1,2]

Sažetak

Posttraumatski stresni poremećaj (PTSP) je kompleksno stanje, uobičajen i onesposobljavajući psihijatrijski poremećaj koji uzrokuje neprocenjivu patnju milionima ljudi. Povezuje se sa visokom stopom funkcionalnih oštećenja, somatskim tegobama, rizikom od suicida i komorbidnih psihijatrijskih poremećaja, kao i sa dodatnim troškovima vezanim za zdravstveni sistem. Postavljanje dijagnoze PTSP-a zahteva dokaz o izloženosti traumi, a karakteriše se simptomima ponovnog doživljavanja traume, izbegavanja i promenama u pobuđenosti i reaktivnosti. Američki sistem klasifikacije dodao je još jedan klaster simptoma koji se odnose na promene u kogniciji i raspoloženju u vezi sa traumom, dok je u evropski sistem klasifikacije dodat složeni PTSP kao nova dijagnoza. Nema dokaza koji podržavaju bilo koju intervenciju kao univerzalnu strategiju prevencije. KBT-TF (usmeren na traumu), KBT i EMDR su pokazali pozitivan efekat. Psihoterapija je prva terapija izbora u lečenju izbora PTSP-a. Preporučuju se intervencije sa fokusom na traumu: KBT-TF, PE i EMDR, kao i terapija upravljanja stresom. Farmakoterapijski pristupi treba da počnu sa jednom od opcija prve linije koja uključuje SSRI kao što su fluoksetin, paroksetin ili sertralin, ili SNRI venlafaksin. Istraživanja koja procenjuju kombinovane psihološke i farmakološke tretmane PTSP-a su ograničena i zahtevaju dalje proučavanje, ali određeni oblici PTSP-a zahtevaju integrativni i multidisciplinarni pristup. Prevencija, rano otkivanje i jasne smernice za lečenje mogu biti najbolji izbor za svaku traumatizovanu osobu, kao i za zdravstveni sistem.

Ključne reči: posttraumatski stresni poremećaj, traumatska iskustva, dijagnoza, farmakoterapija, psihoterapija

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ORIGINAL ARTICLE

Factors associated with the use of health websites and apps among students in medicine and sports

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Summary

Introduction/Aim: The global digital network allows a fast exchange of information. Students of sports and medicine will be the future promoters of healthy behaviors. The study aim was to examine the use of health-related websites and apps among students of sports and medicine in their final undergraduate study year.

Method: This cross-sectional study was conducted at the Faculty of Sports and Physical Education and the Faculty of Medicine, University of Belgrade, Serbia, in the period April-October 2017. A total of 390 students (89 in sports and 301 in medicine) were recruited. Data were collected using an anonymous questionnaire.

Results: Students did not differ in terms of frequency of use of health-related websites (79.8% sport vs. 77.7% medicine; χ^2=0.167, p=0.771) and apps (39.3% sport vs. 49.2% medicine; χ^2=2.672, p=0.116). Fitness and diet were the most common topics that all students sought. A stronger influence of online health information on students' health-related decision-making was associated with the use of health-related websites in both groups of students. Additionally, medical students who used the Internet more often were also more likely to read health-related websites. In terms of apps, being female and using the Internet from a younger age was associated with the use of health apps among sports and medical students, respectively.

Conclusion: Students of sports and medicine equally used health-related websites and smartphone apps. However, health-related websites were much more used compared to health-related apps. Of all health-related topics, they were most interested in fitness and diet.

Keywords: health, internet, apps, students, sports, medicine

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INTRODUCTION

The global digital network allows access to virtually infinite amount of data that are readily available and affordable. This enables fast communication and exchange of information. As a result, health-related information is not any more limited to the conventional sources of information (such as medical books and health care workers), but can be accessible to anyone with the Internet access.

Young adults, including university students, account for the majority of Internet users (1). While some university students have chronic diseases and need specific care (2), most university students are in good health. Empirical data suggest that college students prefer online help rather than physical consultation with a health care professional when they manage their health issues, such as emotional distress (3), as there are numerous websites, health forums and blogs offering helpful information (4). The same holds true for smartphone apps, that can be a useful tool in the efforts to promote healthy lifestyles (5-7).

Although some studies suggested that users of health-related apps were more likely younger people, who are overall in good health and have a higher level of education and higher income (8), few studies explored differences in the field of study and the likelihood of using online health information. For example, a study including French university students suggested that those individuals who study literature/social sciences and life/health sciences read health-related websites and have health-related apps more often than students in technology or law and economy (9). While more frequent use of health-related websites and apps among students of health sciences is obvious and expected, there has been no comparison of use of health-related websites and apps between students who study different aspects of health, such as students of sports and students of medicine.

Theoretical considerations

Several theoretical frameworks and models are applicable to the context of the effective health information communication to optimize health status. These include Social Cognitive Theory, Theory of Reasoned Action/Theory of Planned Behavior, Transtheoretical Model of Change and Health Belief Model. These models take into account individual, relational, communal and societal factors.

The Social Cognitive Theory is based on three essential elements: behavior, intrinsic (personal) factors and extrinsic (outside, environmental) events (10). It highlights that having knowledge (i.e. attention, retention and reproduction) does not necessarily lead to a change in behavior. In fact, it needs to include the intrinsic motivation for change as well as the confidence in one's skills to accomplish that change. In the context of health education, delivery of accurate information is the baseline upon which individuals should further develop their skills through practice that has to be self-driven.

The Theory of Planned Behavior (11), which stems from the Theory of Reasoned Action (12), is related to the notion that behaviors cannot be changed unless there is an intention to change them. However, the intention to change is influenced by individual norms (i.e. acceptance of specific behavior as a preventive strategy), attitudes toward that specific behavior (i.e. how this behavior influences the process of achieving desired outcomes) and behavioral control (i.e. not practicing certain behaviors to reduce the exposure to risk factors). These factors combined lead to the desired behavior through the behavioral intention.

The Transtheoretical Model of Change i.e. Stages of Change Model (13) consists of several sequences, pertaining to both cognitive and emotional mechanisms that are necessary to push behavioral changes forward and keep the new behaviors consistent. These sequences include pre-contemplation (e.g. having an idea what behaviors may be desirable), contemplation (e.g. having the intention to engage in certain behavior), preparation (e.g. allocating time, effort and resources), action (e.g. engaging in certain behavior) and maintenance (e.g. adoption and consistency in behavior in future). This model assumes that the action involves building skills to successfully negotiate health risks and benefits.

The Health Belief Model (14) is based on individual perception of severity of the problem, susceptibility to this issue, perceived benefits and perceived barriers. Through this model, it is possible to define factors which encourage young people to engage in health-promoting behaviors.

Because of the importance and value of years spent in good health, students of sports and medicine will be future promoters of healthy behaviors. We hypothesized that students do not differ in terms of use of health-related websites and apps. The aim of this study was to examine the use of health-related websites and apps, and assess factors associated with its use among students of sports and medicine in their final study year.

METHODS

This cross-sectional study was organized at the Faculty of Sports and Physical Education and the Faculty of Medicine, University of Belgrade, in the period April-October 2017. We recruited students who were in the last year of undergraduate schooling (4th year at the Faculty of Sports and 6th year at the Faculty of Medicine).

The sample size was calculated when the following data were considered: the target population size of 4,000 (approximately 3.500 students in medicine and 500 students in sports), prevalence of health websites use of 50%, margin of error of 5% and confidence interval of 95%. The minimum sample size was 351 students.

The recruitment of students from both faculties was performed as follows: at the Faculty of Sports and Physi-

cal Education, all 4thyear students were divided in five groups, with each group having classroom seminar once a week. Three of the five groups, with approximately 30 students, were randomly selected for distribution of questionnaires. At the Faculty of Medicine, all 6thyear students were divided to five classrooms, all five days of the working week. Three out of the five classrooms were randomly selected each day. There were approximately 20 students per classroom. The questionnaire was filled in at the beginning of the classes. All the students who were offered to fill in the questionnaire agreed to participate in the study, however, 2 students handed in invalid questionnaires (response rate 99.3%). Ethical approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, University of Belgrade. The consent for participation was implied by handing in the completed questionnaire.

Instrument

Anonymous questionnaire was used to collect data. The first segment of the questionnaire examined the participants' socio-demographic characteristics (gender, age, parental marital status, parental highest education attainment, household monthly income, residence prior to entering university, grade point average-GPA, age at the time when they first used the Internet).

Students were asked whether or not they used the Internet and how frequently. We also asked the students whether or not they used health-related websites. A total of 12 most common health topics was listed, based on previous studies (15-17) (fitness and sports, nutrition and diet, sexually transmitted diseases, alcohol and drugs, mental health, partner violence, cancer, sex, cigarettes and tobacco, medications, family violence and bullying). Students were able to choose as many topics as they liked. Additionally, we asked them to report on the most frequently used online resources when searching for health-related content as previously reported (17) (Google, websites run by doctors, Wikipedia, Health portals, health forums and communities, health columns of online media, social networks, websites of health institutions, health blogs, YouTube, Website of Ministry of Health).

We asked the students whether or not they used smartphones and health-related apps, such as fitness/sports, diet/food, water intake, menstrual cycle or other. Lastly, we asked the students to what degree the pieces of health-related information that they find online influenced their health-related decision making.

Data analysis

We used mean values and percentages to describe the study sample. To test the differences between categorical variables, such as the use of health-related websites and apps, we used the Pearson's Chi-square test. To examine factors associated with the use of health-related websites and health-related apps in sports students and medical students, we tested two multivariate logistic regression models. In one model the dependent variable was the use of health-related websites (yes/no) and in the other model the dependent variable was the use of health-related apps (yes/no). In both models, the independent variables (risk factors) were: gender, age, GPA, parental marital status, parental education level, household monthly income, residence prior to studying at the University, age at the time when they first used the Internet, frequency of Internet use and extent to which online health information influences students' decision making.

Statistical significant level was considered that of $p<0.05$. Statistical analysis was performed in SPSS 20.0 statistical software package (SPSS Inc, Chicago, IL, U.S.A.).

RESULTS

A total of 390 University students were included in our study: 89 from the Faculty of Sports and 301 from the Faculty of Medicine. All participants used the Internet. Demographic characteristics as well as patterns of digital health information use are presented in **Table 1**. Students of the two faculties did not differ in frequency of the use of health-related websites (79.8% among sports students and 77.7% among medical students; $\chi^2=0.167$, $p=0.771$) and apps (39.3% among sports students and 49.2% among medical students; $\chi^2=2.672$, $p=0.116$).

The interest in health topics slightly differed between the two groups of students **(Figure 1)**. However, in both groups, students were most commonly interested in fitness and diet. Students of sports did not browse the Internet in search of topics such as partner violence, bullying and tobacco. The distribution of health information sources for our students is presented in **Figure 2**. Students of sports did not report the use of health blogs and websites run by health institutions. Compared to medical students, students of sports more often used health forums and YouTube.

The vast majority of students had smartphones (370, 94.9%). Of those, 183 (49.5%) used health apps. The use of fitness ($\chi^2=0.240$, $p=0.624$), diet ($\chi^2=3.520$, $p=0.061$) and water intake ($\chi^2=0.918$, $p=0.338$) apps did not differ according to the type of faculty. Of female sport students who had smartphones, 41.4% (12/28 students) used menstrual apps and of female medical students 31.0% (62/190 students) used this app ($\chi^2=1.247$, $p=0.264$).

Regression models

After testing the multivariate logistic regression models among sports students and among medical students, we observed several factors associated with the use of

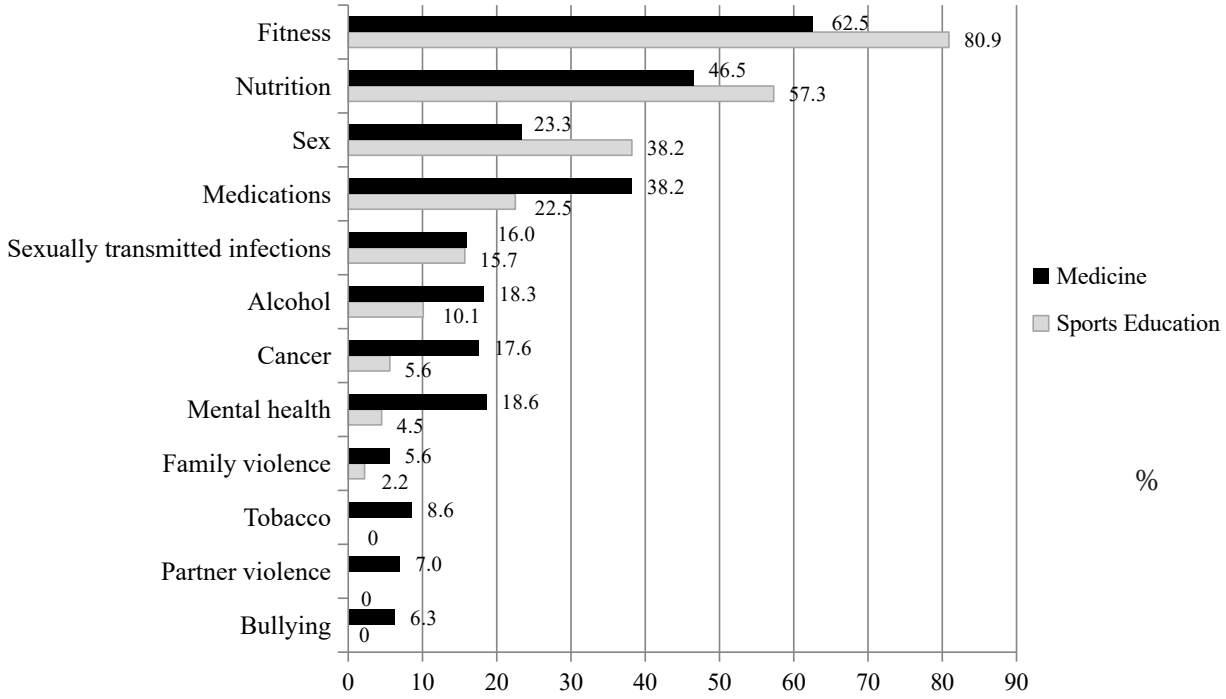
Table 1. Characteristics of the study sample according to the use of health-related websites (N=390)

Variable	Count	Percentage
Gender		
Male	161	41.3
Female	229	58.7
Age (years)*	23.9	1.0
Type of Faculty		
Sports	89	22.8
Medicine	301	77.2
Grade point average*	8.8	0.7
Parental marital status		
Married	327	83.8
Other	63	16.2
Highest education attainment of the mothers		
Primary	11	2.8
Secondary	150	38.5
University	229	58.7
Highest education attainment of the fathers		
Primary	3	0.8
Secondary	160	41.0
University	227	58.2
Household monthly income (Euros)		
< 405	100	25.6
405-810	146	37.4
>810	137	35.1
Missing	7	*1.8*
Residence prior to University		
Capital city	161	41.3
Outside of capital city	229	58.7
Age at first internet use (years)*	13.0	2.8
Frequency of internet use		
Rarely	3	0.8
Once a week	1	0.3
Multiple times per week	10	2.6
Multiple times per day	376	96.4
Use of health-related websites		
Yes	305	78.2
No	85	21.8
Use of smartphone health-related apps		
Yes	183	46.9
No	207	53.1
Extent to which online health-related information influences decision making		
Not at all		
A little	35	9.6
Partially	114	31.7
A lot	193	52.9
Quite a lot	16	4.4
Missing	5	1.4
	27	*6.9*

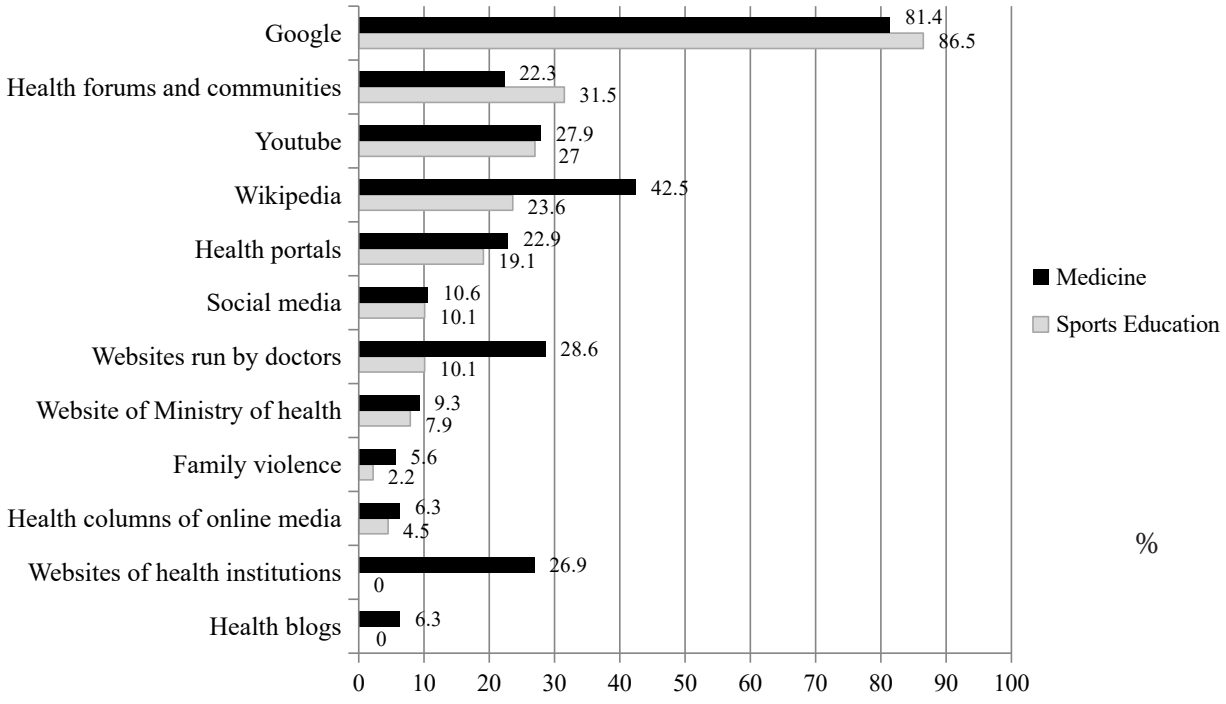
Legend: *Mean value with corresponding standard deviation in brackets; Grade point average range 6.0-10-0

health-related websites and apps. The stronger influence of online health information on students' health-related decision making was associated with the use of health-related websites among both groups of students **(Tables 2 and 3)**. Additionally, medical students who used the Internet more frequently were also more likely to read health-related websites **(Table 3)**.

In terms of apps, being a female was the predictor of health apps use in the group of sports students **(Table 2)**, but this was not observed among medical students. In fact, the only predictor of health apps use among medical students was being younger when they began using the Internet **(Table 3)**.



Graph 1. Distribution of health topics of interest to students according to faculty



Graph 2. Distribution of health information resources for adolescents who visit health-related websites

DISCUSSION

Our findings supported the hypothesis that sport students and medical students did not differ in terms of health websites and apps use. Moreover, their interests in health-related topics were similar. More than three quarters of students in our study reported seeking health information on the Internet. Similar prevalence was observed worldwide, such as among college student in the United States (18), university students in Ireland (19) or Ghana (20). Use of health-related websites was quite high among French University students, accounting for 94.8% in the past year (9).

In our study, both students of sports and medicine were interested in healthy lifestyles, such as fitness and

Table 2. Factors associated with the use of health-related websites and apps among students of sports and physical education in Belgrade, Serbia

Variable	Use of health-related websites		Use of health-related apps	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender Female vs. male*	0.61 (0.15-2.38)	0.472	0.26 (0.09-0.79)	**0.018**
Age range (years)	1.60 (0.74-3.44)	0.230	1.31 (0.80-2.14)	0.287
Grade point average	1.63 (0.54-4.88)	0.382	1.10 (0.44-2.77)	0.934
Parental marital status Married* vs. other	0.59 (0.12-2.95)	0.511	1.10 (0.31-3.95)	0.879
Highest education attainment of the mothers	0.70 (1.95-2.48)	0.576	0.46 (0.15-1.42)	0.178
Highest education attainment of the fathers	0.80 (0.24-2.70)	0.719	2.00 (0.64-6.20)	0.230
Household monthly income	0.57 (0.235-1.36)	0.317	1.11 (0.54-2.24)	0.778
Residence prior to studies Capital city* vs. outside	0.33 (0.08-1.36)	0.124	0.64 (0.22-1.89)	0.423
Age at first internet use	1.03 (0.79-1.33)	0.882	1.12 (0.90-1.41)	0.311
Frequency of internet use	n/a	n/a	n/a	n/a
Extent to which online health-related information influences decision making	2.52 (1.17-5.44)	**0.019**	0.74 (0.40-1.39)	0.356

Legend: OR-odds ratio; CI - Confidence interval; n/a - not applicable; *reference categories; Bold values refer to variables that were associated with the use of health-related websites and apps

nutrition, as expected. A similar pattern was observed among other university students worldwide (9, 21-23). Aside from diet, students in France were most interested in pain and illnesses and mental health (9). Medical students in our study also sought information on medications, which could reflect their field of study, especially drug interactions. A study from the Unites States reported that 81.3 % of students from the University of Illinois reported seeking information on medical treatment, aside from topics on healthy lifestyle (21). It has been observed that the focus on more specific health topics emerges when the perception of individual's own risk increases, such as the case of compliance to seasonal flu vaccination among medical students (22). Finally, the discrepancy in topics of interest between sports and medical students was observed for partner violence, bullying and tobacco, as none of the sport students reported browsing these themes. This may be due to the content worked through in classes, but also due to personal experiences.

Google and Wikipedia were most common platforms browsed by medical students, while aside from Google, sports students used online health forums and online communities. Wikipedia and general health websites were most commonly reported among French university students, as well as the official websites of health institutions (9), which is in line with the reports of our medical students. While it has been argued that Wikipedia is not a credible source of information, a study of quality of its health content and referent sources suggested that Wikipedia offered accurate data which can be used in health care settings (23). Compelling data suggest that Wikipedia could be used as a reliable source of health-related information (24). In fact, a scoping review of literature documented that Wikipedia could be used by the general population just as well as students or health professionals (25), because pieces of relevant information are well-organized, succinct and explained in comprehensible manner. Data from Wikipedia offer information on incidence and prevalence of diseases, risk factors and prevention, all of which are relevant for health education and decision-making regarding health (26). Although health-related content on social media accounted for 10% of users in our study, it has been proposed that social media in the so-called digital age represent an important medium for accessing health information among young adults (27). Furthermore, the increase in health knowledge has been observed after using social health networks for lay public as well (28).

We observed that a factor associated with the use of health-related websites among both sports and medical students was a stronger influence of online health-related information on individual's decision making relative to their health. This finding suggests that online content could play a crucial role in the efforts to increase knowledge on various health topics and provide possibility to better understand and improve personal health behaviors, which could, in turn, help future health professionals in their work with clients and patients. Additionally, in our study using the Internet more frequently was also associated with the use of health-related websites among medical students. Dobransky and Hargittai (21) also observed that students who spent more time on the Internet are more inclined to seek for health information online. This could be explained by being more skilled at browsing the online content after spending more hours on the Internet (21). A number of studies documented that being female was associated with using health-related websites (9, 22, 29), which was not the case among sports and medical students in our study. A potential explanation for not

Table 3. Factors associated with the use of health-related websites and apps among medical students in Belgrade, Serbia

Variable	Use of health-related websites		Use of health-related apps	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender Female vs. male*	1.14 (0.56-2.33)	0.715	0.83 (0.48-1.45)	0.523
Age range (years)	0.69 (0.48-1.01)	0.055	1.22 (0.89-1.66)	0.211
Grade point average	0.71 (0.42-1.20)	0.205	1.11 (0.74-1.68)	0.615
Parental marital status Married* vs. other	1.10 (0.40-3.01)	0.853	0.75 (0.36-1.59)	0.458
Highest education attainment of the mothers	1.27 (0.65-2.50)	0.481	1.51 (0.89-2.56)	0.129
Highest education attainment of the fathers	1.52 (0.71-3.23)	0.276	0.77 (0.43-1.39)	0.390
Household monthly income	0.89 (0.51-1.55)	0.670	0.99 (0.65-1.52)	0.976
Residence prior to studies Capital city* vs. outside	0.65 (0.30-1.38)	0.261	0.99 (0.56-1.73)	0.966
Age at first internet use	1.00 (0.87-1.15)	0.995	0.86 (0.78-.95)	**0.004**
Frequency of internet use	2.57 (1.28-5.14)	**0.008**	0.76 (0.40-1.42)	0.383
Extent to which online health-related information influences decision making	1.82 (1.19-2.79)	**0.006**	1.07 (0.77-1.48)	0.683

Legend: OR-odds ratio; CI - Confidence interval; *reference categories; Bold values refer to variables that were associated with the use of health-related websites and apps

finding a gender difference in health-related websites use could be related to the field of study, as both sports science and medicine explore health from different aspects.

Female gender, however, was observed as a factor associated with the use of health-related apps among sports students. It has been established that women tend to pay closer attention to their health compared to men (30). Moreover, some apps target women specifically, such as follow-up of one's menstrual cycle. Similar findings were observed among university students in France (9), but also in the general population aged 45-64 years (8). In a subset of medical students, the only factor associated with the use of health-related apps was being younger when using the Internet for the first time. This finding supports the notion that young adults who are 'digital natives' are likely to use various digital mediums in search of health-related content. The use of health apps has been reported to improve adults' lifestyle, i.e. it influences an increase in fruit and vegetable intake, and supports physical activity and weight loss (8). Nevertheless, the use of health-related apps among sports and medical students in our study was almost two times less common than the use of health websites. Our data suggest that, relative to digital health information, the use of health-related websites is prevailing compared to health-related apps.

Regarding study limitations, we have recruited students from one university, located in the capital city, which is the largest urban area in the country. The inclusion of other sports and medical faculties in the country could have yielded a larger sample size and an insight into whether the use of health-related websites and apps differed according to urban areas within the country. In the assessment of factors associated with the use of apps on fitness and diet, we have not incorporated students' body mass index, attitudes towards healthy eating habits, physical appearance or overall physical exercise, which could particularly differ between these two groups of students. Inclusion of these characteristics could have helped determine more closely the students who actively used health apps. Additionally, we did not investigate a potential presence of chronic diseases, because adolescents who suffer from certain chronic conditions may be more likely to use apps that support personal management of their condition. Finally, because of a cross-sectional study design we could not make definite inferences between the examined socio-demographic variables and the outcomes of interest.

In conclusion, students of sports and medicine equally use health-related websites and smartphone apps. However, health-related websites were much more used compared to health-related apps. Of all health-related topics, the interest was highest in the fields of fitness and diet. Students whose decision-making was strongly influenced by online health information were more likely to use health-related websites. Female students of sports were more likely to use smartphone apps.

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Author contributions

TG contributed to study design, data collection, data analysis and interpretation and drafted the manuscript. JC, MC, RZ and AP contributed to study design, data collection, data analysis and interpretation and provided critical review of the intellectual content of the manuscript. AG contributed to study design, data analysis and

interpretation and provided critical review of the intellectual content of the manuscript. All authors approved the final version of the manuscript before submission.

Ethical approval

Ethical approval for the study was obtained from the Institutional Review Board of the Institute of Epidemiology, Faculty of Medicine, University of Belgrade.

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PREDIKTORI KORIŠĆENJA SAJTOVA I APLIKACIJA O ZDRAVLJU MEĐU STUDENTIMA MEDICINE I SPORTA

Bojana Pejušković[1,2]

Sažetak

Uvod/cilj: Globalna digitalna mreža omogućava brzu razmenu informacija. Studenti sporta i medicine će biti lideri promocije zdravog ponašanja u budućnosti. Cilj ove studije bio je ispitivanje upotrebe veb sajtova i aplikacija o zdravlju među studentima sporta i medicine na završnoj godini studija.

Materijal i metode: Ova studija preseka sprovedena je na Fakultetu sporta i fizičkog vaspitanja i Medicinskom fakultetu Univerziteta u Beogradu u periodu april-oktobar 2017. godine. Ukupno 390 studenata (od toga 89 studenata sporta i fizičkog vaspitanja i 301 student medicine) je bilo uključeno u studiju. Podaci su prikupljeni korišćenjem anonimnog upitnika.

Rezultati: Ispitanici se nisu razlikovali u pogledu učestalosti korišćenja veb sajtova o zdravlju (79,8% sport prema 77,7% medicina; χ^2=0,167, p=0,771) i aplikacija (39,3% sport prema 49,2% medicina; χ^2=2.672, p=0.116). Fitnes i ishrana su bile najčešće teme koje su svi učenici pretraživali na internetu. Jači uticaj zdravstvenih informacija sa interneta na donošenje odluka u vezi sa zdravljem bio je povezan sa korišćenjem veb sajtova o zdravlju u obe grupe ispitanika. Pored toga, studenti medicine koji su češće koristili internet takođe su češće čitali sadržaj veb sajtova o zdravlju. U pogledu aplikacija, ženski pol i započinjanje upotrebe interneta u mlađem uzrastu su bili prediktori upotrebe aplikacija u vezi sa zdravljem među studentima sporta i medicine, redom.

Zaključak: Studenti sporta i medicine podjednako su koristili veb sajtove vezane za zdravlje i aplikacije za pametne telefone. Međutim, veb sajtovi u vezi sa zdravljem su se mnogo češće koristili u poređenju sa aplikacijama. Od svih tema vezanih za zdravlje, najveće interesovanje je bilo za fitnes i ishranu.

Ključne reči: zdravlje, internet, applikacije, studenti, sport, medicina.

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ORIGINAL ARTICLE

Non-adherence of primary care pediatricians in Serbia to the latest guidelines for the management of acute and chronic cough in children

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Summary

Introduction: Cough is the most frequent symptom in children and can indicate various pathological conditions. Several international guidelines have been published with a purpose of standardizing and improving the assessment and management of cough. Non-adherence to guidelines often leads to a suboptimal quality of patient care. Our aim was to investigate the adherence of Serbian primary care pediatricians to the latest international guidelines on cough diagnosis and management.

Methods: The study included pediatricians working in primary health care centers in Serbia. They were given a questionnaire designed by a team of pediatric pulmonologists which included questions about the choice of diagnostic and therapeutic approaches in acute and chronic cough.

Results: Most of primary care pediatricians assessed the value of international guidelines as fair. However, nearly 70% stated that they prescribed herbal syrup for acute cough. Chronic cough is treated mostly with ivy extract, bronchodilators and antihistamines. A majority of doctors declared positive opinion of herbal medicaments and negative opinion of alternative medicine. About 73% stated that they did not use the chest x-ray as a part of diagnostic procedure.

Conclusion: Serbian pediatricians do not adhere to international guidelines in relation to diagnosis and treatment of cough. The reasons for non-adherence to guidelines should be further investigated.

Keywords: cough, guidelines, children, primary health care

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INTRODUCTION

Cough is an important defense mechanism that helps to clear foreign materials and secretions from airways (1). There are two types of cough depending on duration: acute, which usually lasts for less than three weeks, and chronic, which can last for more than eight weeks (2). Cough is one of the most frequent symptoms in children and one of the most common reasons due to which parents require medical attention for their child (3, 4). Coughing has a major impact on child's sleep, normal daily activities (eating and going to school) and ability to play (5, 6). It has relatively small impact on the child's quality of life, but the members of the child's family are affected by stress and tiredness (7). Annual costs for over-the-counter (OTC) medications for cough are over one billion dollars (8). However, these expenses are underestimation of the global cost of cough management considering the fact that they do not include the cost of the prescribed treatment, physician fees, radiographs, and laboratory testing (9).

Many international guidelines have been published with a purpose of standardizing and improving the assessment and management of cough (10). Since cough can be the indication of various pathological conditions that can progress and raise complications, finding and treating the underlying causes is very important (11). Primary care physicians treat a considerable number of pediatric patients with cough often relying on their established long-term practice rather than on the available guidelines. Insufficient attention has been given to studying the adherence of primary care physicians to guidelines for clinical management of cough.

The aim of this study was to investigate the adherence of Serbian primary care pediatricians to the latest international guidelines on cough diagnosis and management.

METHODS

A multicenter, observational survey was conducted on 615 pediatricians working in primary health care centers located in eight districts (out of 29) in Serbia. The centers were selected based on the pool of pediatricians and regional distribution. The survey was performed from May 2021 to April 2022. The adherence of primary care pediatricians to guidelines on cough diagnosis and management was estimated based on the questionnaire designed using a two-step Delphi method by a group of tertiary care pediatric pulmonologists (12). The study was an anonymous survey and did not require the consent of the Bioethical Committee. Pediatricians were asked to assess the value of four international guidelines related to the diagnosis and choice of therapy of cough in children (13-16). They were also asked which medications they prescribed most commonly for acute and chronic cough, if they used chest x-ray as part of diagnostic procedure, what their opinion was of herbal medicine, and if they advised the use of alternative medicine. The questionnaire consisted of seven questions **(Table 1)**.

Data were analyzed with SPSS IBM Statistics v23 software and expressed as percent values. Distribution of frequencies in the predefined categories for each question were tested by $\chi 2$ analysis. A p-value less than 0.05 was considered statistically significant.

RESULTS

The study participation accounted for 615 pediatricians employed in primary health care centers located in eight districts (out of 29 in total) in Serbia. Of all distributed surveys, 491 (79,83%) were properly completed and returned.

The value of international guidelines for the diagnosis and treatment of cough was assessed as relevant, with 88.89% for treatment and 90.8% for diagnostic procedures, which represents a statistically significant difference compared to the group that rated the guidelines as poor ($p < 0.05$). There were only small differences in the assessment of guidelines regarding the diagnosis and the choice of therapy **(Figure 1)**. Most of the doctors (73%) stated that they did not use the chest x-ray as part of the

Table 1. Format of the questionnaire on cough diagnosis and management

QUESTION	OFFERED ANSWERS
assess the value of four international guidelines related to the diagnosis of cough in children	poor; fair; good; excellent
assess the value of four international guidelines related to the choice of therapy for cough in children	poor; fair; good; excellent
most prescribed medication type for acute cough	nasal decongestants and bronchodilators; antitussives; inhalation drugs; symptomatic drugs; anticholinergics; mucolytics; antibiotics; antihistamines; herbal syrup
most prescribed medication type for chronic cough	antitussives; corticosteroid; expectorants; bronchodilators and antihistamines; antacids; pastilles for sore throat; ivy extract; mucolytics; antibiotics; montelukast; inhalation drugs; beta agonists
the use of chest x-ray as a part of diagnostic procedure	yes; no
opinion about herbal medicaments	positive; negative
the use of alternative medicine	yes; no

diagnostic procedure by algorithms, so when compared with the recommended algorithm for that diagnostic procedure this was significantly lower (p-value <0.01). The dominant medicine for acute cough was herbal syrup, which was prescribed by 68.33 % of pediatricians, and ivy leaves extract EA 757 was most commonly used (65%). Ivy extract was prescribed significantly more frequently compared to any other medication (p <0.05). Each of the other drugs represented less than 10% - in total they participated with 31.67%.

Chronic cough in children was most often treated by ivy leaves extract EA 575 (34%), inhaled corticosteroids (31.4%) and bronchodilators beta 2 agonists (29%). Other drugs were prescribed by less than 15% of pediatricians, in 26.9% cases in total (Figure 3). Approximately 65% of pediatricians declared positive opinion of herbal medicaments. However, more than a half of all doctors (57%) stated that they did not use alternative medicine.

DISCUSSION

This study was designed to determine general principles of managing acute and chronic cough by primary care pediatricians in Serbia with regard to the international guidelines for diagnosis and management of cough. The study showed that non-adherence to guidelines was quite common among Serbian primary care pediatricians, although the majority of them considered the value of international guidelines as fair, both for diagnosis and the choice of treatment. It is a well-known phenomenon that doctors adopt clinical practice guidelines to varying degrees (17). Several factors have been found to influence this. Physicians are concerned about economic motives in designing guidelines or if generalization in guidelines will affect individual patients' needs. Moreover, they usually do not have enough time for reading guidelines in detail. It is also time-consuming to test if new recommended drugs are working or have side effects (16). Most physicians included in this study shared positive opinion of herbal medicines and there was a widespread agreement among them that herbal syrup should be prescribed for acute cough. Although none of the guidelines used in this study mention acute cough, there are quite a few studies proving that herbal drugs are beneficial against it, leading to reduced frequency and severity of cough (13-15, 18,19). Herbal syrup was also found to reduce cough severity in upper respiratory tract infections, which often cause acute cough (20). A smaller number of physicians prescribe antibiotics for acute cough, which is in accordance with guidelines and some other studies (21). Ivy extract is the most prescribed drug for chronic cough. International guidelines do not recommend the use of herbal medicines either for chronic or for acute cough. Despite this fact, ivy extract has already been shown to decrease chronic cough and to be safe for children (21). A significant proportion of pediatricians declared that they prescribed bronchodilators and antihistamines for chronic cough. Bronchodilators are recommended for children with chronic cough in asthma, and antihistamines are recommended for different types of chronic cough which can occur due to infection or allergic rhinitis (13,14). Regarding the use of chest x-ray as part of the examination procedure, about three quarters of physicians claimed that they did not use it. It should be noted that this attitude is present in both acute and chronic cough. A chest radiograph should be used in case of acute cough only under certain circumstances, for example when there is uncertainty about the diagnosis of pneumonia or if there is a possibility of inhaled foreign body, while it is recommended to be always used in chronic cough (23). More than a half of pediatricians included in the study stated that they did not recommend the use of alternative medicine. A few studies showed that many patients used alternative medicine against cough, but only a small number of doctors considered it effective (24,25).

CONCLUSIONS

This study provides an insight into assessment and management of cough in children in primary health care of a developing country. Serbian pediatricians were found not to adhere to the international guidelines in relation to diagnosis and treatment of cough, although they assessed the value of guidelines as fair. The reasons for non-adherence to guidelines should be further investigated among Serbian pediatricians, in order to identify the obstacles to implementing the international guidelines for cough management.

Authors' contributions

Study design: KM,SR. Acquisition: KM,DP,SR. Analysis:KM,SR. Data interpretation: KM,SR. Preparing the draft version of the manuscript: KM,DP,SR. Revising the manuscript: KM,DP,SR.

Ethical approval

This research and publication were approved by the Ethical committee of the University children's hospital (approval number 026 10/02).

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NEPRIDRŽAVANJE PEDIJATARA PRIMARNE ZDRAVSTVENE ZAŠTITE U SRBIJI NAJNOVIJIH UPUSTAVA ZA LEČENJE AKUTNOG I HRONIČNOG KAŠLJA KOD DECE

Katarina Milošević[1,2], Davor Plavec[4] , Snežana Rsovac[3,2]

Sažetak

Uvod: Kašalj je najčešći simptom kod dece i može ukazivati na različita patološka stanja. Objavljeno je nekoliko međunarodnih smernica sa ciljem da standardizuju i poboljšaju procenu i lečenje kašlja. Nepoštovanje uputstava često dovodi do neoptimalnog kvaliteta nege pacijenata. Naš cilj je bio da istražimo pridržavanje srpskih pedijatara primarne zdravstvene zaštite najnovijim međunarodnim smernicama o dijagnostici i lečenju kašlja.

Metode: Istraživanjem su obuhvaćeni pedijatri koji rade u domovima primarne zdravstvene zaštite u Srbiji. Dobili su upitnik koji je osmislio tim dečjih pulmologa a koji je uključivao pitanja o izboru dijagnostičkih i terapijskih pristupa kod akutnog i hroničnog kašlja.

Rezultati: Većina pedijatara primarne zdravstvene zaštite ocenila je vrednost međunarodnih smernica kao iskoristljivu. Međutim, skoro 70% je izjavilo da propisuje biljni sirup za akutni kašalj. Hronični kašalj se leči uglavnom ekstraktom bršljana, bronhodilatatorima i antihistaminicima. Većina lekara se izjasnila pozitivno o biljnim lekovima, a negativno o alternativnoj medicini. Oko 73% njih je izjavilo da ne koristi rendgentski snimak grudnog koša kao deo dijagnostičke procedure.

Zaključci: Pedijatri u Srbiji u primarnoj zdravstvenoj zaštiti se ne pridržavaju u potpunosti međunarodnih smernica u vezi sa dijagnostikom i lečenjem kašlja. Razloge za nepridržavanje smernica treba dalje istražiti.

Ključne reči: kašalj, smernice, deca, primarna zdravstvena zaštita

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ORIGINAL ARTICLE

Brain natriuretic peptide as a predictor of clinical outcome and symptom improvement after a left ventricular assist device implantation

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The authors have declared that no competing interests exist

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Summary

Introduction: The predictive value of brain natriuretic peptide in heart failure is well-known, but its importance as an outcome predictor after a left ventricular assist device (LVAD) implantation remains unaddressed. This research aims to examine the significance of brain natriuretic peptide (BNP) as an indicator of treatment outcomes during different post-implantation periods.

Methods: A retrospective cohort study included the analysis of medical records of 87 patients in whom LVAD was implanted at the University Clinical Center of Serbia in the period 2014-2020. The correlation of BNP levels with treatment outcomes after operation was evaluated.

Results: The average preoperative BNP for all patients was 1244 pg/mL. The average ejection fraction (EF) was 15% (median), endsystolic diameter (ESD) was 6.80 cm and endiastolic diameter (EDD) was 7.70 cm. Eighty-one patients were NYHA class 4, and 6 patients were NYHA 3. Compared to the preoperative BNP level below or above 1000 pg/mL, there was no significant difference in overall survival of patients after operation (Log Rank [Mantel-Cox] test, p=0.838). BNP levels postoperatively decreased from 358 pg/mL at 3 months upon surgery to 136 pg/mL 5 years upon surgery (Friedman test, p<0.001). BNP levels show strong negative correlation with EF (decrease in BNP level and increase in EF value), and strongly positive correlation with EDD, ESD, and NYHA class (a decrease in BNP and a decrease in EDD, ESD, and NYHA).

Conclusion: Preoperative BNP level may not be an adequate predictor of the outcome after LVAD implantation, but the post-implantation decrease in BNP levels is highly correlated with parameters that indicate an improvement in the clinical status and positive changes in the myocardium and long-term survival.

Keywords: LVAD, BNP, heart failure, reverse remodeling, NYHA

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INTRODUCTION

Implantation of a left ventricular assistance device (LVAD) is a widely used method of treatment of end-stage heart failure (HF). It was primarily conceived as a bridge to transplant (BTT) device but was later approved for use as definitive (destination) therapy (DT). (1) The most current publications show survival rates suggesting that the LVAD implantation program can become an alternative to heart transplantation (HTx), otherwise the gold standard in the treatment of HF, considering the inherent limitation of the HTx concept based on the lack of donors and increasingly good results of LVAD implantation (2).

Brain natriuretic peptide (BNP) is secreted primarily by ventricular myocytes as a reactive response to myocardial load and damage. BNP was first isolated in the brain tissue, but it was later proven that the ventricular myocardium is the primary site for the release of this hormone. Increased BNP levels can help clinicians to differentiate HF and monitor the effects of various treatments, stratify the risk after acute ischemic coronary events, and monitor possible cardiotoxicity during chemotherapy, but the final conclusions are supplemented by other clinical signs and examinations (3). Many reports on the prognostic value of BNP in HF have been published but reports on the importance of BNP in the clinical follow-up of LVAD patients are significantly scarcer in terms of content and number (4).

The aim of the paper is to use a retrospective analysis to examine the correlation of brain natriuretic peptide (BNP) level with clinical outcome after LVAD implantation and with anatomical and functional changes in the left ventricular myocardium in the post-implantation period.

MATERIAL AND METHODS

A multidisciplinary cardiology and cardiac surgery team performed a retrospective analysis of the medical records of patients in whom an LVAD device was implanted at the University Clinical Center of Serbia from January 2014 to December 2020. Demographic and clinical data from the pre-implantation period, data from surgery as well as follow-up data (histories, echocardiographic findings, and laboratory results) were collected for the following time points: before implantation, 30, 90 and 180 days post implantation, one year upon implantation, and for every subsequent post-implantation year. Possible unscheduled and additional follow-up visits were analyzed depending on the reason for the visit/hospitalization or adverse events and/or complications.

BNP levels were determined in the samples using chemiluminescent immunoassay, CLIA method, using Atellica IM, Siemens. The test is a double sandwich immunoassay that uses direct chemiluminescent technology based on constant amounts of two monoclonal antibodies. The first antibody is a mouse monoclonal antibody to human BNP, labeled with acridinium ester, characteristic of the ring structure of BNP. The second solid phase antibody is a biotinylated mouse monoclonal anti-human antibody characteristic for the C-terminal part of BNP, bound to streptavidin magnetic particles. After the reaction, there is a direct relationship between the amount of BNP in the sample and the relative light units (RLUs) detected by the system. The analytical sensitivity of the test is 2 pg/mL. The reference level (95th percentile) is <100 pg/mL.

The primary endpoints for analysis were patient survival and a decrease in heart failure symptoms, a change in New York Heart Association (NYHA) class, a change in the left ventricular ejection fraction (EF), changes in left ventricular end-systolic (ESD) and end-diastolic diameter (EDD).

Complete statistical analysis was performed with the statistical software package IBM SPSS version 26.0. Attribute variables are presented as the frequency of certain categories, numerical variables are presented as mean values with standard deviation or median with interquartile range (25-75th percentile), depending on the normality of the data distribution, which was tested by the Kolmogorov-Smirnov test. Patient unadjusted survival was calculated using Kaplan -Meier plot (mean value with 95% confidence interval) and the value obtained with the Log Rank (Mantel-Cox) test. Spearman's rank correlation was used to assess the relationship between the observed parameters and BNP levels. All analyses were assessed with the $p<0.05$ level of statistical significance.

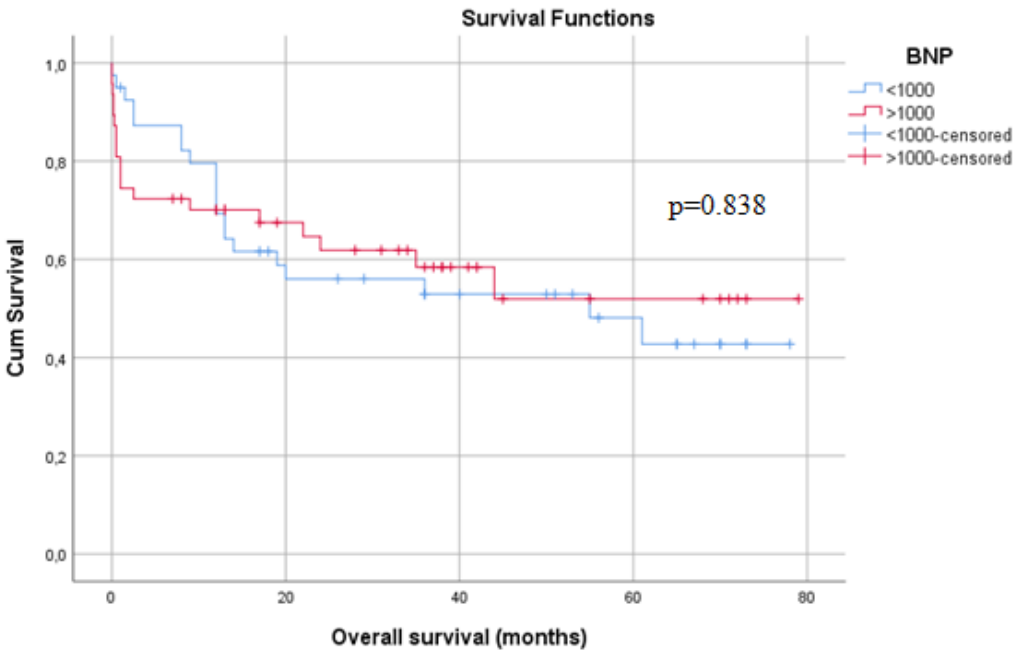
RESULTS

The study included 87 patients implanted with an LVAD pump from 2014 to 2020. **Table 1** shows basic

Table 1. Preoperative demographic and clinical characteristics of patients

Gender: Male/Female	79 (90.8) / 8 (9.2)
Age (years)	54.75 ± 12.06
BNP (pg/mL)	1244 (577-2500)
BNP: <1000 / >1000	40 (46.0) / 47 (54.0)
BTT / Definitive therapy	61 (70.1) / 26 (29.9)
NYHA: III / IV	6 (6.9) / 81 (93.1)
INTERMACS class: 1-2 / 3-4 / 5	27 (31.0) / 52 (59.8) / 8 (9.2)
eGFR (mL/min/1.73 m^2)	60 (44-60)
Pump type: Heart Mate II / Heart Mate III / HeartWare	30 (34.5) / 24 (27.6) / 33 (37.9)
Creatinine (μmol/L)	108 (77-142)
Duration of hospital stay (days)	25 (20-31)
Outcome: survival/death	48 (55.2) / 39 (44.8)
EF (%)	15 (12-20)
ESD (cm)	6.80 (6.05-7.45)
EDD (cm)	7.70 (6.70-8.40)

Results are presented as number (%), mean ± standard deviation or median with interquartile range (25-75th percentile).

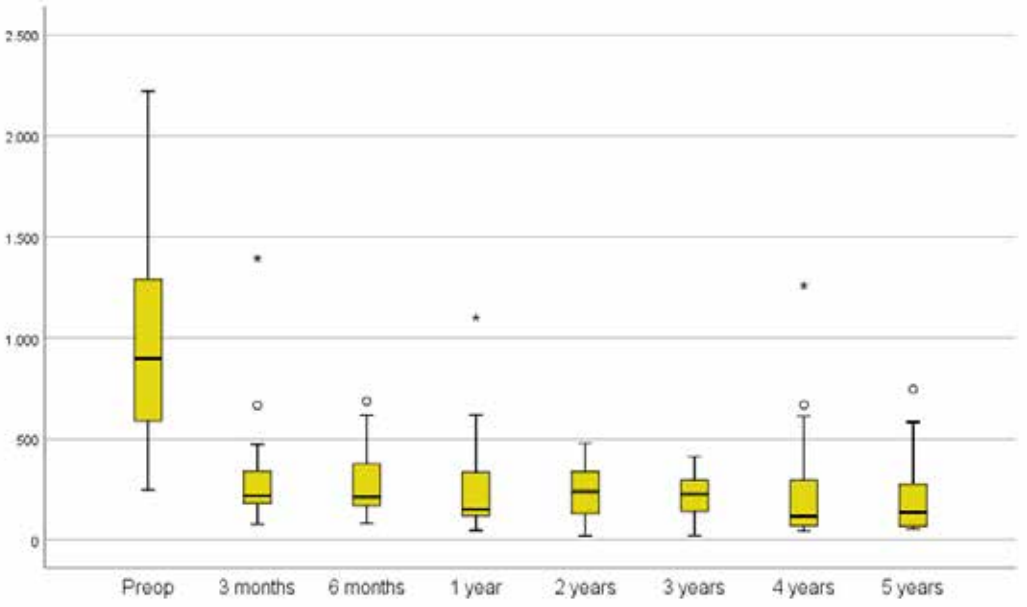


Graph 1. Overall survival relative to BNP level (p=0.838)

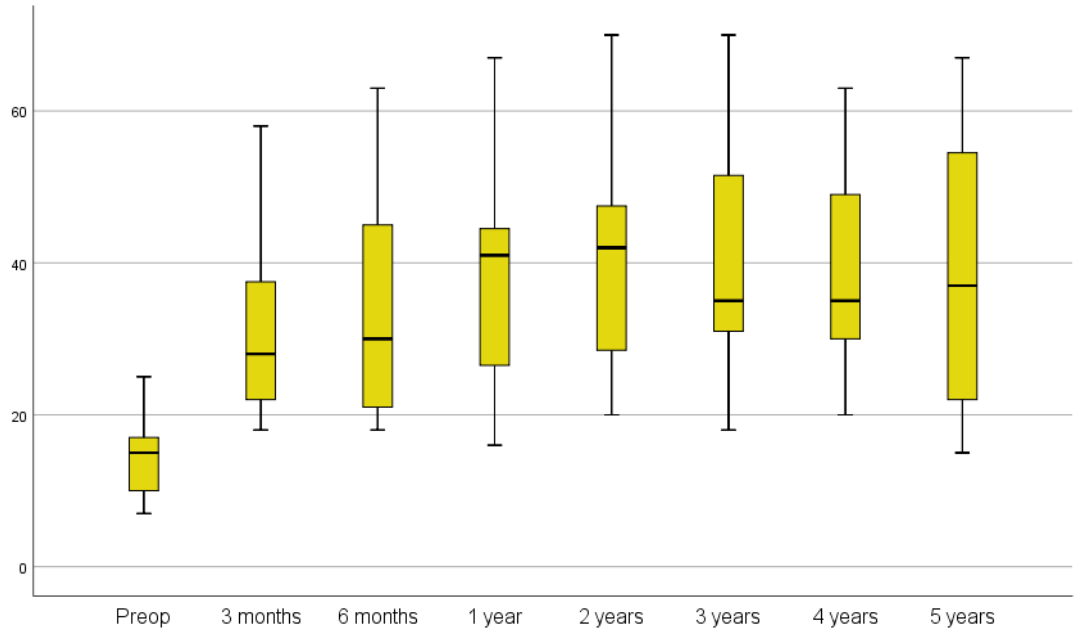
preoperative characteristics of these patients. Most of them were male, with an average age of about 55 years. All patients were classified as NYHA class III and IV. The average ejection fraction was 15% (median), while ESD was 6.80 cm and EDD 7.70 cm. The average preoperative BNP level for all patients was 1244 pg/mL. Survival rate after 5 years was 43%. For the purpose of statistical analyses, we divided the patients into two groups based on the preoperative BNP level; there were 46% of patients with BNP levels below 1000 pg/mL and 54% of patients with BNP levels above 1000 pg/mL.

Relative to BNP level (>1000 pg/mL vs. <1000 pg/mL), no significant difference was found in the overall survival after LVAD implantation (Log Rank [Mantel-Cox] test,). **Graph 1** shows that, in the first 20 months, mortality was higher in the group with preoperative BNP level >1000 pg/mL, while after that and until the end of the follow-up period, mortality was higher in the group with preoperative BNP level <1000 pg/mL. The average survival rate was 47.19 months (95% confidence interval: 36.27-58.11) in the group with preoperative BNP level >1000 pg/mL, and 44.44 months (95% confidence interval: 34.09-55.00) in the group with BNP level <1000 pg/mL.

Graph 2 first shows preoperative and postoperative BNP levels during the period of 5 years upon surgery. Preoperative levels had already been shown to be significantly higher with an average of 1244 pg/mL, while postoperative levels improved, showing a decrease from



Graph 2. BNP levels (pg/mL) preoperatively and at 5 years upon surgery



Graph 3. EF values (%) preoperatively and at 5 years upon surgery

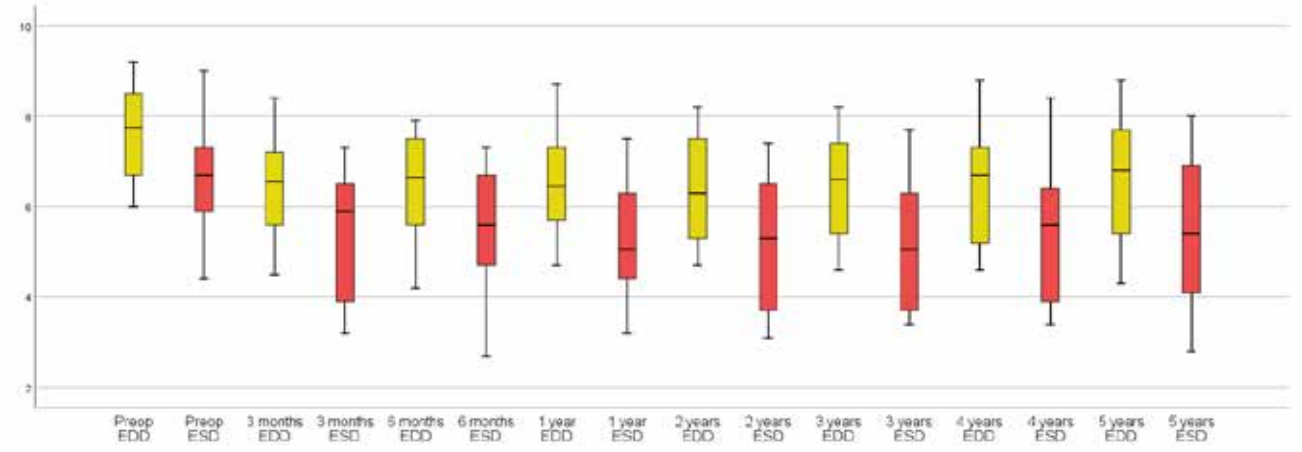
358 pg/mL at 3 months after surgery to 136 pg/mL 5 years upon surgery (Friedman test, p<0.001).

Graph 3 shows preoperative and postoperative EF values during the period of 5 years after surgery. Preoperative value had already been shown to be significantly lower at an average of 15%, while postoperative value improved, showing an increase from 30% at 3 months upon surgery to 37% 5 years upon surgery (Friedman test, p<0.001).

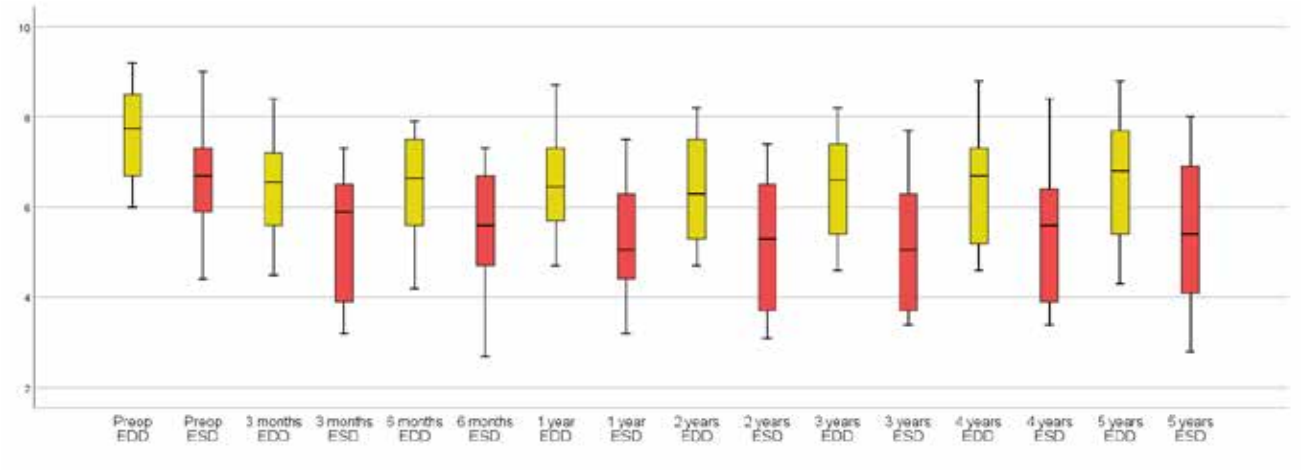
Graph 4 shows preoperative and postoperative EDD and ESD values preoperatively and postoperatively during the period of 5 years after surgery. Preoperative ESD value had already been shown to be significantly higher at an average of 6.80 cm, while postoperative value improved, showing a decrease from 5.80 cm at 3 months after surgery to 4.90 cm at 5 years after surgery (Friedman test, p<0.001). Preoperative EDD value was significantly higher at an average of 7.70 cm, while postoperative value improved, showing a decrease from 6.55 cm at 3 months after surgery to 6.80 cm at 5 years upon surgery (Friedman test, p<0.001).

Before surgery, most patients were NYHA class IV **(Graph 5)** and the rest were NYHA class IIIb. However, at 3 months after surgery, the relationship changed with most of them being NYHA class II, while at 5 years after surgery most of them were NYHA class I, suggesting that the long-term survival of these patients is highly correlated with the degree of HF symptom reduction.

By analyzing the association of BNP levels with several most significant parameters that were monitored before and after surgery **(Table 2)**, we observed a significant negative association between preoperative BNP levels and EF. BNP levels at 6 months are associated with NYHA class only, while BNP at one year is strongly negatively associated with EF (a decrease in BNP level and an increase in EF value), and strongly positively associated with EDD, ESD and NYHA class (a decrease in BNP level and a decrease in EDD, ESD and NYHA category



Graph 4. EDD and ESD values (cm) preoperatively and at 5 years upon surgery



Graph 5. Number of patients by NYHA class, preoperatively and at 5 years upon surgery

Table 2. Association of BNP with EF, EDD, ESD, and NYHA class

BNP	EF	EDD	ESD	NYHA
Preoperatively	r=-0.278 p=0.009*	r=0.173 p=0.109	r=0.144 p=0.189	r=0.079 p=0.464
After 3 months	r=-0.171 p=0.160	r=0.228 p=0.059	r=0.162 p=0.184	r=0.219 p=0.071
After 6 months	r=-0.142 p=0.250	r=0.212 p=0.083	r=0.218 p=0.074	r=0.353 p=0.003*
After 1 year	r=-0.320 p=0.012*	r=0.445 p<0.001*	r=0.427 p=0.001*	r=0.471 p<0.001*
After 2 years	r=-0.396 p=0.009*	r=0.181 p=0.245	r=0.255 p=0.099	r=0.501 p=0.001*
After 3 years	r=-0.370 p=0.029*	r=0.349 p=0.040*	r=0.355 p=0.040*	r=0.602 p<0.001*
After 4 years	r=-0.344 p=0.127	r=0.317 p=0.162	r=0.340 p=0.132	r=0.070 p=0.764
After 5 years	r=-0.660 p=0.007*	r=0.424 p=0.115	r=0.474 p=0.075	r=0.371 p=0.173

Sperman's rank correlation; *- p<0.05

values). BNP levels after 2 years showed the association with NYHA class and EF similarly to those after year 1, and again after 3 years in the same way with all monitored variables. After 5 years, BNP levels remained strongly negatively associated to EF only.

DISCUSSION

Our research showed that all patients who are candidates for LVAD implantation have increased BNP levels several times above the reference levels, which is consistent with end-stage heart failure. Considering that the reference level of BNP is less than 100 pg/mL, and that all patients had a preoperative level far higher than normal (even several tens of times higher), it did not prove to be a parameter that can provide a prognosis of survival immediately after implantation, even at levels >1000 pg/mL which, according to some publications, is the value that categorizes NYHA class IV end-stage heart failure. Over a longer post-implantation period, the BNP level showed a significant decline compared to pre-implantation levels and stood in correlation with patient long-term survival. Also, a decrease in BNP level is correlated with observed echocardiographic changes, an increase of EF, and a decrease of ESD and EDD, as well as with an improvement in NYHA class. This suggests a reverse process remodeling of the myocardium after LVAD implantation, i.e., recovery of myocardial function at the level of heart ventricles and BNP serum levels as predictors of such positive physiological-anatomical myocardial changes.

Natriuretic peptide system includes three elements. A-type, the atrial natriuretic peptide mostly synthesized in the atria in response to stress of this part of the heart. C-type is produced in the brain, pituitary gland, kidneys, and endothelial cells, but generally at low serum concentrations. Ventricles initially secrete prohormone called pro BNP, which is then transformed by enzymatic processing into NT-proBNP, which consists of 76 amino acids and BNP with its 32 amino acids. These two proteins are isolated from serum in clinical practice, but the concentration of NT-pro BNP is 3 to 6 times higher than the concentration of BNP due to its longer half-life (5,6). The basic cardioprotective mechanism of this peptide is the reduction of volume load by reducing the concentration of salt and water in the body and by vasodilation. Basically, BNP is an antagonist to renin-angiotensin-aldosterone cardiovascular hormonal cascade that is released in response to a decrease in atrial pressure (7).

In addition to pathological conditions that cause myocardial strain, serum BNP levels are also increased in diabetes, acute coronary damage, and kidney disease. It is important to note that the BNP level is lower in people with obesity. Also, BNP level increases depending on the patient age, sex (women tend to generally have higher levels than men), and there are daily variations in the level in many patients. Therefore, the assessment of the clinical cause of BNP level disorders and its predictive value

should be assessed taking into consideration the individual characteristics of the patient (8). A level of 100 pg/mL is usually taken as the cut-off value that discerns cardiac from non-cardiac causes of dyspnea (9). BNP levels progressively increase as the patient's NYHA category increases, so the levels above 1000 are usually present in heart failure with NYHA IV category and are characteristic of patients with pronounced symptoms of end-stage heart failure (10,11).

As discussed above, the number of studies specifically dealing with BNP as a predictor of outcome after LVAD implantation is small. Sato et al. showed the significance of a decrease in BNP levels after LVAD implantation concluding that such a trend was associated with long-term patient survival. A significant number of previous publications point out that the greatest recovery of cardiac function occurs in the period up to 60 days upon surgery, and especially after the first post-implantation month. The mentioned period represents the interval in which the reverse myocardial remodeling reaches its maximum. The results of our study are generally in agreement with this conclusion (12).

Yost et al. in their publication state that 14 days is the optimal time point for determining the level of BNP decrease and subsequent interpretation of such decrease as an outcome predictor (13).

Papathanasiou et al. in their research reported a decrease in BNP levels after LVAD implantation, but with levels that are still high. They emphasize the importance of using other biomarkers and considering non-cardiological parameters to obtain relevant conclusions related to the predictive role of BNP (14).

In addition to the proven increase in the survival rate and an improvement in quality of life compared to non-surgical methods of treatment of end-stage heart failure, LVAD patients are exposed to the risk of various post-implantation complications. These include, but are not limited to infections, hemorrhages in various organs, the right ventricle weakness, pump thrombosis, and cerebrovascular insult (15). In their research, Hegarova et al. tested a hypothesis that elevated levels and changes in BNP concentration in LVAD patients correlated with the occurrence of adverse events. The increase in BNP levels due to post-implantation adverse events is thought to be primarily due to increased ventricular filling pressure and stress on ventricular myocytes. This study suggested special, individual BNP levels that can potentially warn and correlate with certain adverse events and complications, but these conclusions were not confirmed by larger studies (16).

Methods of LVAD implantation were described in many publications and are part of regular cardiac surgical practice. The input cannula is placed in the top part of the left ventricle, the output graft is connected to the aorta, and the energy for work is obtained through an external (extracorporeal) source that is connected to the device via a drive line (drawn through the skin of the abdomen). After implantation, LVAD device takes over the action of the left ventricle and increases the systemic circulation flow. Subsequently, this leads to a significant increase in the inflow to the right ventricle (this is the reason why the right ventricle must have sufficient contractile reserve), which responds to this with increased work and pulmonary circulation flow. At the same time, faster and more efficient emptying of the left ventricle reduces the load in the pulmonary circulation. Such a hemodynamic cascade, along with an additional improvement of kidney function in most patients, leads to a major unloading of the myocardium at the level of both ventricles and, subsequently, to a decrease in BNP synthesis.

The improvement of myocardial function that has been damaged by certain cardiac pathology after surgical or non-surgical treatment, with the correction of previously occurring pathoanatomical changes, is defined in literature as "reverse myocardial remodeling" (17,18). There is a significant number of reports that point to the unequivocal conclusion that the LVAD implantation leads to such positive changes that also affect the outcome of treatment. Reverse LV remodeling after LVAD implantation has been associated with a decrease in plasma BNP levels. Reduced synthesis of BNP has been documented even in samples of myocardial cells (19).

CONCLUSION

LVAD implantation significantly alleviates symptoms of heart failure with accompanying positive changes at the anatomical and physiological level of the myocardium. Preoperative BNP level is not an adequate predictor of survival after LVAD implantation. Postoperative BNP levels, i.e., a drop in BNP levels after implantation is correlated with long-term patient survival accompanied by an increase in EF, a decrease in EDD and ESD, as well as changes in the NYHA category.

LIMITATIONS

The conclusions were based on the study performed at a single cardiac surgery center. The analysis was performed on 87 patients, which represents a relatively small number of subjects. This research has limitations typical of other retrospective, non-randomized studies.

Ethical Statement: Conducting such a retrospective study is in accordance with the provisions of Helsinki Declaration and was approved by the Ethics Committee of the University Clinical Center of Serbia.

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MOŽDANI NATRIURETSKI PEPTID KAO PREDIKTOR KLINIČKOG ISHODA I POBOLJŠANJA SIMPTOMA NAKON UGRADNJE UREĐAJA ZA TRAJNU MEHANIČKU CIRKULATORNU POTPORU

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Sažetak

Uvod: Moždani natriuretski peptid (BNP) potvrdio je svoju prediktivnu vrednost u stanju srčane insuficijencije, ali njegov značaj kao prediktora ishoda nakon ugradnje uređaja za trajnu mehaničku cirkulatornu potporu (LVAD) nije obrađivan u dovoljnoj meri da bi se izveli nedvosmisleni zaključci. Cilj istraživanja je da ispita značaj BNP-a kao parametra koji može ukazivati na ishode lečenja u različitim postimplantacionim vremenskim periodima.

Metode: Učinjena je retrospektivna kohortna studija analizom medicinske dokumentacije 87 bolesnika kojima je LVAD uređaj ugrađen u Univerzitetskom Kliničkom Centru Srbije od 2014. godine do 2020. godine. Analizirana je korelacija nivoa moždanog natriuretskog peptida sa ishodima nakon operativnog lečenja.

Rezultati: Preoperativni BNP je u proseku bio za sve pacijente 1244 pg/ml. Ejekciona frakcija (EF) je u proseku bila 15% (medijana), endsistolni dijametar (ESD) 6,80cm, a enddijastolni dijametar (EDD) 7,70cm. 81 bolesnik pripadao je NYHA klasi 4, a 6 bolesnika NYHA klasi 3. U odnosu na to da li je vrednost BNP preoperativno bila manja ili veća od 1000 pg/ml nije nađena značajna razlika u ukupnom preživljavanju pacijenata nakon ugradnje LVAD pumpe (Log Rank (Mantel-Cox) test, p=0,838). Vrednost BNP –a postoperativno se smanjivala od 358 pg/ml 3 meseca nakon operacije do 136 pg/ml 5 godina nakon operacije (Friedman test, p<0,001). Vrednosti BNP negativno je izrazito povezan sa EF (pad vrednosti BNP-a, a rast vrednosti EF), a pozitivno izrazito povezan sa EDD, ESD i NYHA klasom (pad vrednosti BNP-a i pad vrednosti EDD,ESD i kategorije NYHA).

Zaključak: Preoperativni BNP nije adekvatan prediktor ishoda nakon LVAD ugradnje, ali postimplantacioni pad vrednosti BNP-a visoko korelira sa parametrima koji ukazuju na poboljšanje kliničkog statusa i pozitivnim promenama na miokardu i dugoročnim preživljavanjem.

Ključne reči: LVAD, BNP, srčana slabost, reverzni remodeling, NYHA

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ORIGINAL ARTICLE

Attitudes and opinions of first year medical students regarding peer-assisted learning of histology and embriology: the role of undergraduate teaching assistents

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Summary

Peer-assisted learning (PAL) is a form of teaching method where students facilitate the transfer of knowledge to other students and at the same time learn themselves. Department of Histology and Embryology at the Faculty of Medicine, University of Belgrade, Serbia has a longstanding tradition of PAL, which involves the use of undergraduate teaching assistants (UTAs) in practical microscopy classes. This research aimed to evaluate the attitudes and opinions towards UTAs work during Histology and Embryology course from the perspective of 1st year students. Data from this study were collected from 512 undergraduate medical students enrolled in the 1st study year using an anonymous semi-structured questionnaire. The analysis of the students' responses has shown that almost all of our students asked for the help of UTAs during Histology and Embryology course. Additionally, 65% of students said that they felt more comfortable asking UTAs for help during their labs than faculty teachers. Most students believe that UTAs devoted sufficient time to them during the practical classes. Nearly 90% of students agreed that UTAs gave them good advice and answers and more than 80% of students perceived that they had never received inaccurate information from UTAs. More than 40% of students can see themselves in the role of UTAs in future. These results indicate that UTAs have a significant place in the Histology and Embryology curriculum and that their role in everyday teaching represents one of the most important aspects of PAL.

Keywords: peer-assisted learning, medical education, teaching, curriculum

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INTRODUCTION

Peer-assisted learning (PAL) is a well-established teaching method where non-professional teachers, such as students, facilitate the acquisition of knowledge by other students, helping them to master the educational material and at the same time learn themselves, thus undertaking an active role in the process of teaching (1, 2, 3). This form of education has found its application in all levels of education, but nowadays it is most dominant in the field of higher education, especially in medicine, where it has found its educational place in numerous preclinical (4-9), as well as clinical subjects (10-12).

Different forms of PAL can be classified according to its three main components – educational distance, group size and formality (13, 14) – distance being the main factor that distinguishes these educational programs (15). When referring to the difference in educational distance, the term peer tutoring refers to the relationship in which student-teachers and student-learners are in the same academic year, while the term near-peer is used for the relationship where the difference between student-teachers and student-students is at least one academic year (14-16). One of the main forms of near-peer teaching at faculties is implemented using undergraduate teaching assistants (UTAs) (17). Various roles and responsibilities of UTAs have been described in literature (3, 18), as well as their positive benefits to student-learners and themselves in the fields of biomedical sciences (3, 5, 17, 19 - 21).

At the Department of Histology and Embryology, Faculty of Medicine, University of Belgrade, Republic of Serbia, there has been almost a century-long tradition of actively engaging UTAs in practical microscopy classes. Their task is to help 1st year students recognize and analyze histological slides, as well as draw and label given samples and help them with the course material. UTAs at our department are preselected from students who have successfully passed the Histology and Embryology exam with high marks (9/10 or 10/10) and are at least at the 2nd year of their medical studies. Additionally, these students are interviewed by department members to assess their competence and interest in being UTAs. Although throughout the previous decades we have seen many positive effects of UTAs on our 1st year students and Histology and Embryology teaching in general, we haven't had the opportunity to inspect the attitudes and opinions of 1st year students about the UTAs work so far. Therefore, this research aimed to evaluate the quality of work of UTAs at the Department of Histology and Embryology from the perspective of 1st year students and to look into their attitudes and opinions towards this form of near-peer education program.

MATERIAL AND METHODS

Histology and Embryology course

Histology and Embryology is a mandatory, two-semester subject for 1st year medical students at the Faculty of Medicine, University of Belgrade, Republic of Serbia. The course takes 30 weeks, and it is organized into lectures, practical classes (microscopy labs), and seminars or online (blended) activities. Students can choose to have traditional computer classroom seminars, or they can be enrolled in an online course of Histology and Embryology entitled "Reticulum", based on Moodle Learning Management System (LMS). For a detailed description of the Histology and Embryology course at the 1st year of medical studies and the structure and organization of the curriculum at the Faculty of Medicine, University of Belgrade, the readers are referred to our previous paper (25).

Subjects

The present study included a total of 512 undergraduate medical students enrolled in the 1st year of the Faculty of Medicine, University of Belgrade. The survey was done at the end of the second semester and during the final week of microscopy labs in Histology and Embryology, at our department. The study aims were introduced to the participants and each of the students gave their consent to voluntarily and anonymously participate in the survey. The present study was also done as a part of the quality assurance and quality improvement program at the Department of Histology and Embryology, Faculty of Medicine, University of Belgrade.

Instrument

Data from this study were collected using an anonymous digitally semi-structured questionnaire. The questionnaire was comprised of questions regarding demographic characteristics, such as gender, and previous education, as well as the questions and assertions concerning the student's attitudes towards the quality of work of UTAs. The study results are shown in the form of descriptive statistics (frequencies, percentages). One question was in the form of assertion which was rated on a 5-point Likert scale, where answer 1 denoted the attitude "I completely disagree" and answer 5 denoted the attitude "I strongly agree" with the given statement. These results were later dichotomized so that the answers ranked 4 or 5 on the Likert scale were classified as a positive attitude or "agreement" and answers ranked 3, 2, and 1 on the Likert scale were classified as a negative attitude or "disagreement". Additionally, the questionnaire included two open questions, where students were able to express their opinions on what characteristics and virtues make good UTAs and why some of them would like to become UTAs in future.

RESULTS

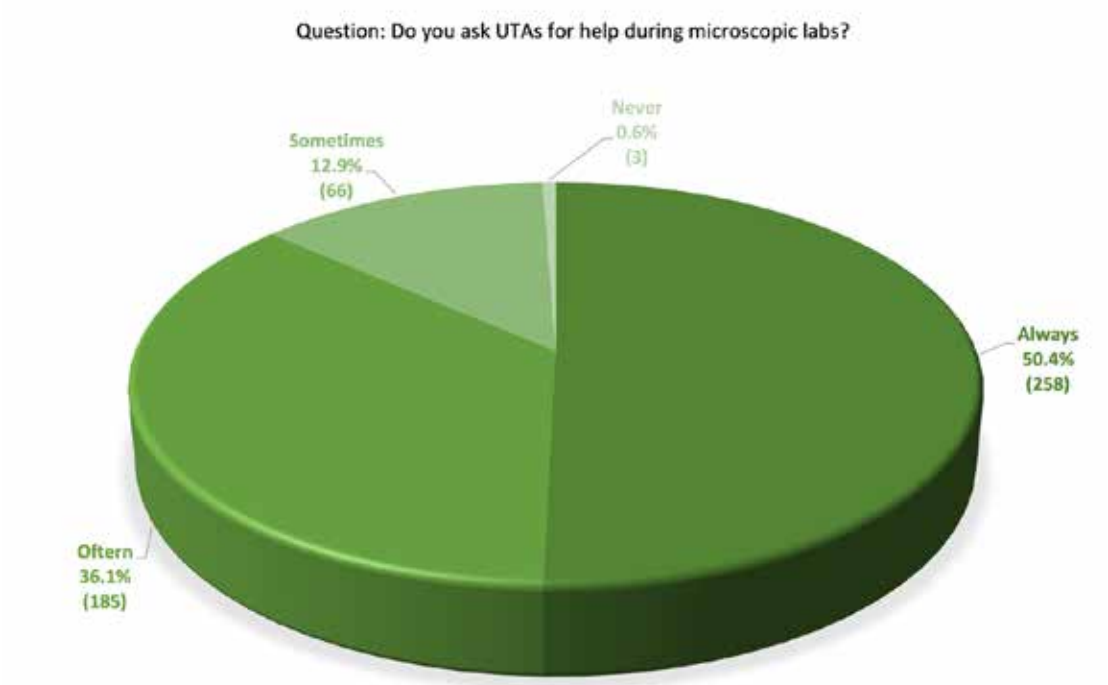
A total of 512 1st year medical students (151 male and 361 female students) participated in the study and completed the semi-structured questionnaire. Most of the students had previous high school (313/512, 61.1%) or secondary medical school education (182/512, 35.5%), while only 3.3% (17/512) students have completed some other type of secondary school.

The Histology and Embryology course has 27 practical microscopy classes during two semesters (13 in the first semester and 14 in the second semester). For UTAs to fulfill their teaching obligations, they must be present in at least 11 microscopy labs during one semester, which is cumulatively about 80% of the total practical classes during the course. When students were asked whether the UTAs regularly attended their microscopy labs, most students responded positively (455/512, 88.9%), while 11.11% (57/512) students reported that UTAs occasionally appeared in their classes. None of the students have responded that UTAs did not attend microscopy labs.

The main role of UTAs in the Histology and Embryology course is to help and teach 1st year students how to properly use a microscope, find adequate elements in histological slides, as well as to help them draw and label those structures in their workbooks. Almost 90% of our students have, to some extent, asked for the help of UTAs during the Histology and Embryology course and less than 1% (3/512) of students have never asked for UTAs help **(Figure 1)**. Additionally, 65% of students (334/512) have said that they felt more comfortable asking UTAs for help during their practical courses than asking faculty teachers (teaching assistants, assistant professors, and associate professors). Only one student (0.2%) has responded negatively about asking both from faculty teachers and UTAs for assistance. We were also interested in seeing which segment of microscopy labs UTAs were most helpful with. The student's answers show that UTAs have a very important role not just in microscopy, analyzing, drawing, and labeling of specimens and structures, but also students turn to UTAs when they have questions concerning the Histology and Embryology teaching material **(Table 1)**.

Most of the student respondents (78.5%, 402/512) believe that UTAs devoted sufficient time to each of them during practical classes. However, a fifth of students (20.5%, 105/512) state that UTAs did this sporadically, while only 5 students (1%) have claimed that UTAs never devoted enough time to them. 1st year medical students have also expressed their thoughts on the quality of answers and advice given by the UTAs in the form of the following statement: *UTAs gave me good advice and provided answers to my questions concerning Histology and Embryology teaching material*. Almost 90% (455/512) of students agreed with the abovementioned assertion, while only 11% (57/512) of students showed some degree of disagreement **(Figure 2)**.

Since UTAs at our Faculty can be students from 2nd to 6th year of study who have successfully passed Histology and Embryology exam, one should be aware that they are nonetheless still undergraduate students and that their knowledge may vary and sometimes be even incorrect. For this reason, we aimed to check if the 1st year students ever had a perception that UTAs gave them false information and if it was so, to what extent. Our analysis has shown that more than 80% (407/504) of students claimed that they had never received inaccurate information



Graph 1. Students' responses to the question if they needed help from the undergraduate teaching assistrants during microscopy classes of Histology and Embriology

about the Histology and Embryology teaching course material. Although this is the majority of students there is a significant number of students (17.5%, 88/504) who have stated that UTAs sometimes gave false or inaccurate information, and this percentage should not be neglected.

Table 1. Students' responses to the question for what reason they asked undergraduate teaching assistants for help during microscopic classes of Histology and Embryology.

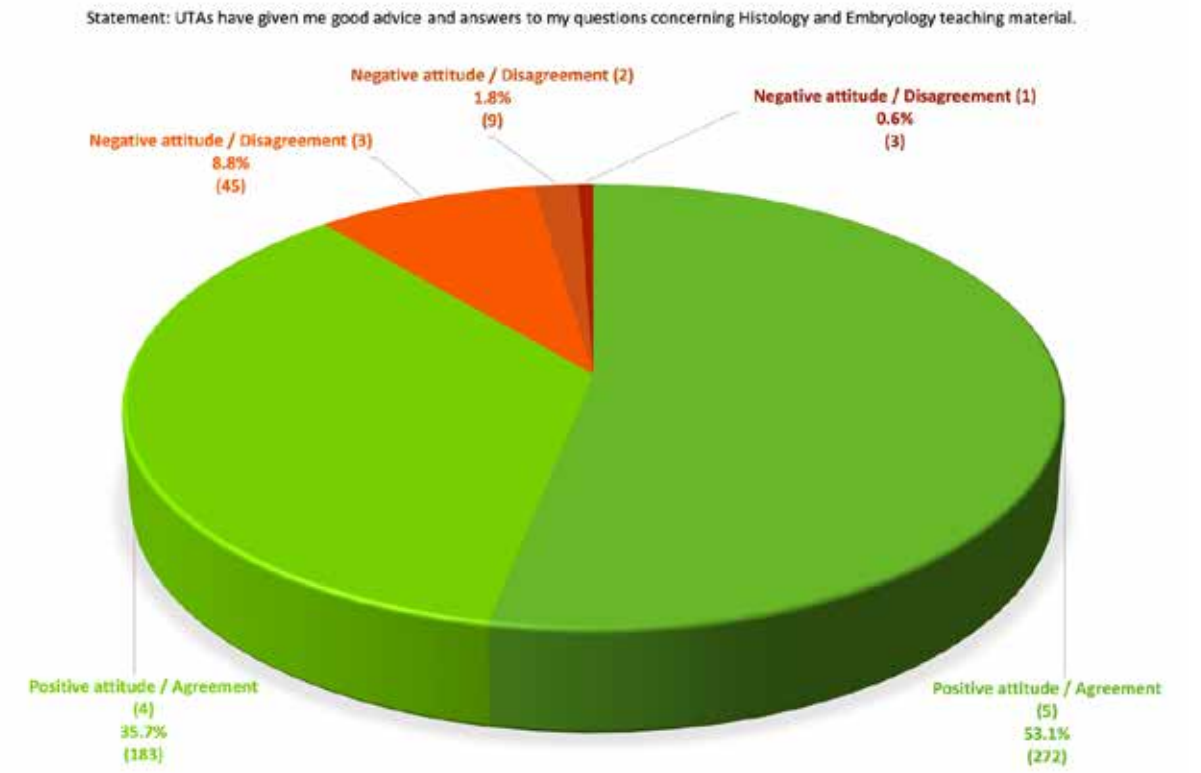
For what reasons do you ask UTAs for help during microscopic labs?	Np/Ntot (%)
I ask for assistance with microscopy and drawing of histological slides.	146/512 (28.5%)
I ask for assistance with microscopy and drawing of histological slides, but also with Histology and Embryology teaching material.	338/512 (66%)
Whenever I ask for help I always have a question concerning Histology and Embryology teaching material.	25/512 (4.9%)
I never ask questions.	3/512 (0.6%)

An interesting result arises as a response to the question in which 1st year students have been asked if they see themselves in the role of UTAs in future. Although a large percentage of students gave a positive answer to this question (42.6%, 218/512), there was a significant number of students who gave a negative answer (31.1%, 159/512) and students who did not have a clearly defined attitude towards this question (26.4%, 135/512). In the final question, students had the possibility to give an open answer as to which personal qualities and characteristics described a good UTA. In **Table 2** we have listed some of the most interesting and striking comments given by our students.

DISCUSSION

When trying to classify the form of PAL present in the Histology and Embryology course at our faculty, and according to the categories proposed by Olaussen et al. (15), we could say that it predominantly belongs to the near-peer tutorial form. The near-peer form of PAL implies that there is a difference of at least one academic year between students, as it is the case between our 1st year students and UTAs (13). Additionally, we can further classify this form of near-peer teaching as near-peer tutoring, since usually one UTA is assigned to 10 students (15). However, in the past year, due to certain legislations that have been adopted by our university, the number of UTAs at our faculty significantly decreased. Thus, we can now define the current situation as near-peer didactic form, where the ratio between UTAs and students exceeds 1:10 (15). According to the environment in which peer education is conducted, our course belongs to the formal form, since it has been implemented in the official curriculum (14, 15).

Among the obtained study results, we can see that almost all our students (86.5%) have always or often asked for the help of UTAs to some extent. Interestingly, 65%



Graph 2. The percentege of students who agreed or disagreed with the given assertion. The 5-point Likert scale answers have been additionally dichotomized, so that answers ranked 4 or 5 have been considered as positive attitude (i.e., agreement) and answers ranked 1, 2 and 3 have been considered as negative attitude (i.e., disagreement)

Table 2. Students' responses to an open-ended question about what describes a good undergraduate teaching assistant

What describes a good UTA?
Having a good attitude towards students, i.e., let them know they can freely ask everything they don't know, even if those questions are related to basic things from a certain area or subject. Of course, a UTA must know the things he/she teaches, but even if they don't, they should be willing to ask other UTAs or teachers for advice and explanation. After all, nobody can know everything.
First, a UTA must have knowledge and secondly they must be able to transfer the knowledge to other students in a way that they feel relaxed and comfortable when they are given help and explanation. A UTA should not be arrogant about their knowledge.
Someone who fully understands new students and therefore knows exactly how to help them.
A good undergraduate teaching assistent is a student who is willing to help other students in the right way regarding the subject matter, and for other medical-related things, such as medical studies in general and teachers. They should have sufficient knowledge of the subject matter for which they are elected, be in a good mood, and should not scare off younger students.
Timeliness, goodwill, good attitude, and the power to transmit knowledge.
A good presentation of certain lessons with guidance on how to learn certain course materials.
Remembering to ask a student if he needs any help and then to give that student enough attention and time for adequate explanation. Trying to clarify, in the best way possible, all of the things that are unclear to students.
Always willing to help and devote enough time to everyone.

Abbreviations: UTA – undergraduate teaching assistant

of them have said they had more freedom to ask UTAs for help than their teachers. Regarding our UTAs' knowledge, most 1st year students (88.9%) replied that they were given good advice and answers, while more than 80% of them claimed that they had never received inaccurate information related to course material. These results could be explained by the theory of cognitive congruence (13, 20, 23 - 25). This theory relies on the assumption that learning process consists of upgrading the already existing prior knowledge in pupils. Additionally, it is known that student-teachers (UTAs in our case) better perceive the Vygotsky zone of proximal development, which represents a difference between what a learner can do with and without appropriate assistance (25, 26). Thus, teachers, who by default possess more knowledge than learners, can show a certain degree of cognitive incongruence, which in turn may distance the students from them (4, 20). On the other hand, due to their ability to realize the zone of proximal development, UTAs are much more cognitively congruent and share more connections with their student learners (5, 13, 14, 20, 25). Therefore, it is possible that student-learners believe that student-teachers who had a recent contact with the same teaching material and have passed the Histology and Embryology exam, may have a lot more understanding for them than the teachers who deal with the given subject matter much longer and on a higher level (20, 27).

A student who wants to be a UTA at our faculty is required to have a good grade point average and to have passed the subject they want to teach to other students with high marks. These students are chosen by faculty members of a certain department and are usually considered to be among best students in a certain subject, who possess similar knowledge, abilities, skills, and higher enthusiasm than graduate teaching assistants (18). Thus, it is not surprising that more than 80% of our 1st year students stated that they had the perception that they had never received inaccurate information from UTAs. However, we should not overlook the fact that more than 17% of students have stated that they received some form of inaccurate information from UTAs. Mistakes are present in every aspect of teaching profession, and in UTAs' work, mistakes could be even more pronounced since they are still students and learning their mistakes is present in everyday aspect of their studies. Medical students are aware that errors in medical education occur from the beginning of their studies, but the question is whether students themselves, and in our case UTAs, are aware of those mistake and wrong information they may have transferred to their student-learners (28).

Trying to evaluate additional aspects of UTAs influence, we also had an open-ended question on what described a good UTA from a student's standpoint. By analyzing the student's comments, we have been able to see the presence of another very important relation, such as social congruence, which also underlies PAL (8, 14, 15, 20). This relation draws on the importance of student-learner and student-teacher relationship, where the student-teacher is acting as a role model. In our situation, the concept relies on the fact that if UTAs have been able to overcome certain difficulties while learning Histology and Embryology, the student-learners will gain confidence that they will also be able to do so. Thus, both groups of students build a mutually rewarding and encouraging relationship, where UTAs improve their skills as educators and enhance confidence, while student-learners are stimulated to work harder and be more involved in the learning process (2, 3, 14, 20, 25, 29, 30). However, one should be aware that near-peer learning changes with the distance between learners and teachers (31). Graduation is thought to be one of the main transitions where social and cognitive incongruence become most apparent, but we now know that this happens even

sooner in the so-called transition zone, which is used to denote senior and last-year students (8).

Being a UTA means having a lot of obligations and duties, but it also brings numerous positive experiences and advantages such as development of teaching, social, communication, and leadership skills, improvement and advancement of current knowledge, and thus the level of self-confidence, as well as the feeling of enjoyment and enthusiasm as a result of being able to participate in someone's education (20, 25, 32 – 34). It seems that students can recognize these positive effects since more than 40% of them answered that they can see themselves in the role of UTAs in future. However, more than 50% of students had a negative opinion about being a UTA or did not have a clearly defined opinion. The reasons for this may be numerous, starting from a lack of interest in Histology and Embryology as a subject, indifference towards participation in the education of other students, or even unawareness of what the job of UTA is like and what the abovementioned positive effects it contains. The last is supported by 26% of students who did not have a defined opinion on whether or not to become a UTA. This highlights the need for formal development of students' teaching skills in an undergraduate curriculum since it can point out many benefits of near-peer learning to students, the principal being - teach others and become better learners (19, 20, 25). Also, both students and UTAs acquire self-confidence and collaborative skills for learning, listening, and discussing as a team (21).

A study by Pintér et al. shows a positive impact of near-peer tutors on student development in basic surgical skills training, suggesting the application of near-peer trainers' technique as cost-efficient and mutually beneficial (35). The benefits of near-peer teaching are also shown in the basic life support course, acute care skill training course, and clinical skills and simulation course (36). Thampy et al. proposed using general practitioners in training as near-peer tutors for medical students, given their eagerness to teach and learn. They also suggest that students in turn value teaching by their near peers (37). In all examples, students stated that they felt more comfortable seeking help and guidance from near-peer teachers and found them more approachable than their regular course teachers.

Our study had several limitations which should be noted, as they may have an impact on result interpretation. The main limitation lies in the distance diversity between our UTAs and 1st year medical students. As noted in the introduction section UTAs can be students who have successfully passed the Histology and Embryology exam and thus can be students from 2nd to 6th year of medical studies at the Faculty of Medicine in Belgrade. Since our 1st year students did not have the information about the UTAs' year of study, we were unable to assess the impact of distance between learners and teachers, which as we are aware, can significantly influence the quality and outcomes of PAL. The second factor is UTAs' regularity of attendance. Each year we have UTAs who do not fulfill their teaching obligations or decide to leave their position, mainly due to other faculty obligations which progressively increase with the year of study. However, according to the answers in our students, this factor did not significantly influence the study results as the majority of students (almost 90%) reported that UTAs regularly attended their microscopy labs.

CONCLUSION

The results of this study shed light on how student-learners were satisfied with the work of UTAs and it is currently the only study that has examined this form of near-peer learning method in Histology and Embryology teaching. Students' responses have shown that they feel more comfortable working and contacting UTAs for assistance and that the information provided by UTAs are correct and useful. According to our students, a good UTA should always be willing to help, devote enough time, have sufficient knowledge, and create comfortable atmosphere where student learners can feel free to ask questions. These results indicate that UTAs have a significant role in Histology and Embryology curriculum and that their role in everyday teaching represents one of the most important aspects of near-peer learning. Also, the impact that UTAs have on student-learners is a sign for us to additionally address their teaching education and to stimulate such students to further develop their teaching skills, from which both student-learners and student-teachers would benefit.

Based on the obtained results we now have two key directions in which future research on UTAs' impact on Histology and Embryology teaching in Serbia can be focused. Firstly, the effects of distance between learners and teachers should be thoroughly studied in the context of how this relationship changes with UTAs' year of study. Additionally, it would be interesting to see if the quality of work of UTAs differs if we consider the information about UTAs' Histology and Embryology education - whether their teachers were UTAs during their studies and if they had any previous pedagogic experience.

The second important direction is directly related to the number of UTAs. Shortly after the completion of this study, the university has made a decision to decrease the number of UTAs in all subjects in which they are engaged. This has led to changes in the PAL towards the near-peer didactic form of teaching. If we were to repeat this research under current conditions, it would be interesting to see if the UTAs' time devoted to 1st year students and thus the quality of their work have significantly changed.

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STAVOVI I MIŠLJENJA STUDENATA PRVE GODINE MEDICINE O UČENJU HISTOLOGIJE I EMBRIOLOGIJE UZ POMOĆ VRŠNJAČKOG PODUČAVANJA: ULOGA STUDENATA DEMONSTRATORA

Miloš Bajčetić[1], Ana Pešikan[2] , Katarina Milutinović[1], Ana Ilijevski[1], Aleksa Leković[3], Jelena Rakočević[1], Ivan Zaletel[1]

Sažetak

Vršnjačko podučavanje (eng. peer-assisted learning, PAL) je vrsta nastavne metode gde učenici prenose znanja drugim učenicima i kroz taj process istovremeno i sami uče. Katedra za histologiju i embriologiju Medicinskog fakulteta Univerziteta u Beogradu ima dugogodišnju tradiciju vršnjačkog podučavanja, koja se ostvaruje angažovanjem studenata demonstratora na osnovnim studijama tokom praktičnih časova mikroskopije. Cilj ovog istraživanja bio je da se iz ugla studenata prve godine procene stavovi i mišljenja o radu studenata demonstratora na predmetu histologija i embriologija. Podaci su prikupljeni od 512 studenata sa prve godine osnovnih akademskih studija medicine korišćenjem anonimnog polustrukturisanog upitnika. Analiza odgovora pokazala je da su skoro svi naši studenti tražili pomoć studenata demonstratora tokom trajanja nastave iz histologije i embriologije. Pored toga, 65% studenata izjavilo je da im je lakše da traže pomoć od studenata demonstratora nego od zvaničnog nastavnog osoblja. Većina studenata veruje da su im studenti demonstratori posvetili dovoljno vremena tokom praktične nastave. Skoro 90% studenata se složilo da su im studenti demonstratori dali dobre savete i odgovore, a više od 80% smatra da nikada nisu dobili netačne informacije od demonstratora. Više od 40% studenata može da vidi sebe u ulozi demonstratora u budućnosti. Ovi rezultati ukazuju da studenti demonstratori imaju značajno mesto u nastavnom planu i programu histologije i embriologije i da njihova uloga u svakodnevnoj nastavi predstavlja jedan od najvažnijih aspekata vršnjačkog podučavanja.

Ključne reči: vršnjačko podučavanje, medicinska edukacija, nastava, kurikulum

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CASE REPORT

Giant porocarcinoma of the leg - diagnostic and thearpeutic chalenges, and possible influence of the COVID-19 pandemic on the diagnosis and treatment

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Summary

Introduction: Cutaneous porocarcinoma is a rare malignant tumor, arising from the eccrine sweat gland. High rates of extracutaneous spread, local recurrence and high mortality are observed. Definitive diagnosis could be complex due to its histopathological similarity to squamous cell skin carcinoma and Paget's disease. Primary treatment still relies on surgical excision.

Patient review: A 76-year-old male presented at our clinic in January 2020, with a bleeding skin tumor of the right thigh. Multiple wide surgical excisions were performed between January 2020 and November 2022, due to repetitive local recurrence. For a period of time, our patient was lost to follow up caused by COVID-19 pneumonia and a long recovery period. Initially, a poorly differentiated squamous cell skin carcinoma was reported. In the first post-COVID-19 follow-up, in January 2022, there was a new tumor locally, and porocarcinoma was diagnosed. In follow-ups, apart from skin tumor recurrence, inguinal nodal involvement was confirmed. CT showed no signs of disseminated disease in the lungs or in the abdomen, in any of the succeeding follow-ups. Finally, MRI of the pelvis revealed a suspicious mass in the bladder, radiologically consistent with metastatic disease and the patient was referred to an oncologist and a urologist.

Conclusion: Porocarcinoma remains insufficiently explored. There are no official treatment protocols. Due to its similarity to squamous cell carcinoma, a dermatopathology expert should be consulted, while a multidisciplinary approach is necessary for managing the disease. Since early diagnosis and treatment are of paramount importance, any factors that delay them, such as COVID-19 pandemic in our case, could be detrimental to the patient and poorly affects the prognosis and final outcome.

Key words: eccrine porocarcinoma, porocarcinoma, skin tumors, COVID-19

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INTRODUCTION

Cutaneous eccrine porocarcinoma is a rare, malignant, non-melanocytic skin tumor, arising from the eccrine sweat gland. It accounts for 0.005% to 0.01% of all skin tumors, with a high rate of extracutaneous spread and high mortality rate. (1,2) In recent literature, less than 500 cases of porocarcinoma have been described since the disease was first described by Pinkus and Mehregan in 1963. (2,3)

The etiology of this tumor still remains unknown while the gender distribution was often found to be equal. (3,4) The average age of onset is in the seventh decade, while the head and neck are the most common sites for tumor occurrence, followed by the lower extremities, and rarely other parts of the body (3). Establishing a definitive diagnosis of porocarcinoma could be complex due to its histopathological similarity to squamous cell skin carcinoma and Paget's disease. Recurrence of the primary tumor occurs in 20% of cases, with 20% of tumors metastasizing to regional lymph nodes, presenting a poor prognostic parameter. The occurrence of distant metastases has also been reported. If lymph node metastases are present, the mortality rate reaches 67% in these patients. (5,6)

Considering the rarity of this skin tumor, official treatment protocols and procedures have not been established, with primary treatment still relying on surgical excision. (2,7)

Here, we present a case of giant, recurrent porocarcinoma of the leg, diagnostic and therapeutic challenges, and possible influence of the COVID-19 pandemic on diagnosis and treatment.

CASE REPORT

A 76-year-old male patient presented at our clinic in January 2020, with a 70x60mm exulcerated skin tumor on the lateral aspect of the right thigh. The patient had no recollection of the tumor's first occurrence but had been aware of the tumor's growth. The patient's decision for a check-up at the doctor's office of the growing skin tumor was based on the occurrence of spontaneous bleeding from the tumor. A wide surgical excision was performed and the defect was reconstructed with two transpositional flaps. Histopathological analysis reported a poorly differentiated squamous cell skin carcinoma, with 14 mm maximal thickness, and clear resection margins. The patient was referred for further, regular radiological and surgical follow-ups every 3 months, but the patient was lost to follow-up.

In further development, the patient reported COVID-19 pneumonia with subsequent sequelae during that period, which was later confirmed by a computerized tomography (CT) scan. The patient also reported a long recovery period.

In January 2022, the patient presented with a large, 270x110 mm tumor recurrence at the site of the previous surgery **(Figure1)**.

A wide local excision was performed and the defect was reconstructed with split-thickness skin grafts from the left femoral region. The histopathological report made by a dermatopathologist revealed porocarcinoma, with 20mm thickness, vascular invasion and positive resection margin. On imunohistochemical analysis, the tumor showed positivity with pan-cytokeratin (AE1/AE3), focal cytokeratin 7 reaction, rare ductal structures with anti-carcinoembryonic (pCEA) antibody, and proliferative index (Ki-67) of 30-40%. CD117 immunostain was negative. Also, revision of the slides from the previously resected tumor confirmed porocarcinoma, instead of squamous cell carcinoma. Another aggressive surgical approach with a wide tumor excision was performed obtaining clinically clear excisional margins, but histopathological evaluation revealed the involvement of the margins in some parts of the specimen. The wound site was reconstructed using split-thickness skin grafts taken from the lateral arm region. The inguinal ultrasound report was unremarkable. The patient was referred to an oncologist for further evaluation of adjuvant treatments.

At the next follow-up, echosonographic report confirmed the advancement of the disease in the right inguinum, as well as loco-regional skin tumor recurrence. Given the suspicion of nodal metastatic disease, a lymph node biopsy with surgical excision of the recurrent tumor was performed. Histopathology of the samples from various parts of the recurrent skin tumor showed typical porocarcinoma **(Figure2)**, except in one sample where a pleomorphic tumor with sarcomatoid morphology (CK-positive) was found. A lymph node biopsy found metastatic deposits in parenchyma as well as in the lymphatic vessels in the surrounding perinodal fat tissue. Computerized tomography (CT) showed no signs of disseminated disease in the lungs or in the abdomen, in any of the subsequent follow-ups.

Figure 1. Surgical treatment of recurrent porocarcinoma: **(a)** large recurrent porocarcinoma of the thigh. **(b)** the patient received multiple surgeries until clear resection margins were obtained. **(c)** soft tissue defect covered with split-thickness skin grafts. **(d)** Final result after surgery

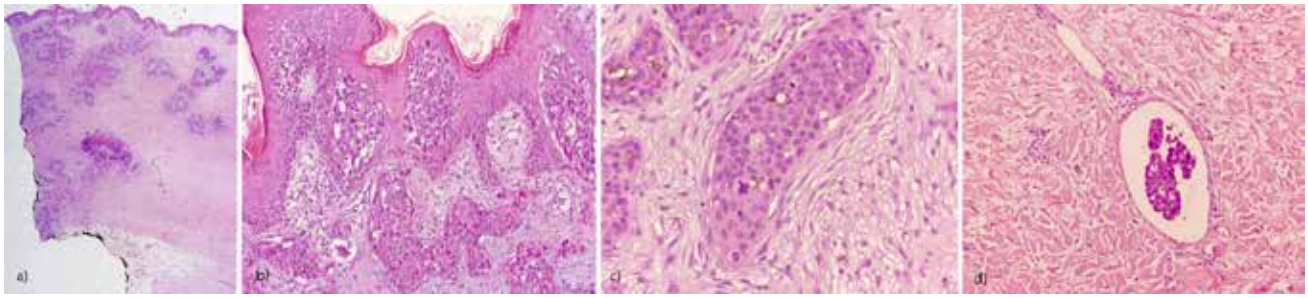

Figure 2. Porocarcinoma histology - **(a)** deeply invasive tumor consisting of anastomosing tumor islands; **(b)** intraepidermal tumor nests (Borst-Jadassohn phenomenon); **(c)** intracytoplasmic lumina and mitotic figures in dermal tumor nests; **(d)** tumor vascular invasion (hematoxylin&eosin; original magnification a x12.5; b,d x100; c x200)

In November, a severe loco-regional tumor recurrence affecting the skin surrounding the grafted surgical site as well as the rest of the leg was clinically observed (Figure3), together with lymphadenopathy of the affected lower extremity. Chest CT showed no metastatic disease. The magnetic resonance of the pelvis revealed a suspicious mass in the bladder, radiologically consistent with metastatic disease, as well as with a possibility of other primary tumors, and the patient was referred to an oncologist and a urologist.

DISSCUSION

Eccrine porocarcinoma is a rare skin tumor developing from the acrosyringium, the terminal, intraepidermal parts of the sweat gland ducts. It is considered to arise de novo, or secondary to a long-term benign eccrine poroma, nevus sebaceous, or actinic. (2,5) One meta-analysis of 463 patients found that the majority of patients presented with poromas prior to tumor malignant alteration, with a latency period as long as 60 years. (3) At the time of diagnosis, the reports of 22.3% of patients with metastatic disease could be found in the literature. (2)

The final diagnosis is established by biopsy and histopathological verification. Often misdiagnosed as squamous cell carcinoma, as reported in literature, immunohistochemical stains are found to be helpful in porocarcinoma verification. Some of the most commonly used stains include carcinoembryonic antigen (CEA), cytokeratin (CK) (pancytokeratin and CK5/6), epithelial membrane antigen (EMA), p53, and p63. (2,8), and CD117. (9) Still, histopathological verification remains the cornerstone for diagnosis, and it sometimes requires an experienced pathologist, as in our case, where the initial diagnosis was squamous cell carcinoma, changed into porocarcinoma after revision by a dermatopathologist. (10) Surgical removal of the tumor by wide local excision is the mainstay treatment to this day, followed by further radiological diagnostics in order to detect possible spread of the disease. (10) Cure rates after wide surgical excision are reported to be 70–80% with clear margins, depending on the histopathological tumor type, while Mohs micrographic surgery showed excellent results in individual cases. (5) Additionally, studies on margins of surgical excision are inconclusive in the context of patient outcomes and recurrence, and further evaluation are needed. (2)

Figure 3. Advanced loco-regional and metastatic disease at the last follow-up. (a) The tumor affecting the entire lower extremity. (b) Tumor recurrence surrounding the site of previous surgeries. (c) Advancement of the tumor to the distal parts of the lower extremity.

Any delay in surgical treatment of patients could lead to poorer prognoses, higher incidence of metastatic disease, and higher mortality rate. (4)

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has significantly affected hospital admissions for non-COVID patients worldwide. Due to the reorganization of the healthcare system during the pandemic, our clinic was among those transformed into COVID-19 hospitals. Following the resumption of the regular work regime, all elective surgeries were suspended on several occasions depending on the national epidemiological situation, while the criteria for hospitalization were rigorous and defined by the National Guidelines, with the aim of reducing the risk of virus transmission among patients and staff. (11) Medical personnel were recruited to specialized COVID-19 hospitals. Additionally, elderly patients were more likely to have avoided going to the doctor fearing the risk of virus transmission, and our patient experienced a serious form of COVID-19 as well, suffering from consequences for a prolonged period. All of these factors, directly or indirectly connected with COVID-19, could contribute to a delay in diagnosis and treatment.

Robson et al. reported three factors to be indicative of a poor prognosis in eccrine porocarcinoma: a tumor depth of more than 7 mm, a high mitotic count (> 14 / high-power field), and vascular invasion. (8) Advanced disease signifies a poor prognostic parameter associated with high mortality, leading to survival periods ranging from 5 to 24 months. (1)

Sentinel lymph node biopsy (SLNB) is a successful tool in identifying occult lymph node metastasis with 81.3% positivity rate. (2) The recommendations for SLNB include high-risk features such as tumor depth of more than 7mm, with or without palpable lymphadenopathy, as well as tumor localization. Tumors of the head and neck are found to rarely metastasize, while in the event of other localizations, a high suspicion index could be justified.

Recurrence of the primary tumor occurs in 20% of cases, with 20% of tumors metastasizing to regional lymph nodes, presenting a poor prognostic parameter. (12) In case of confirmed metastases in the regional lymph nodes, dissection of the regional lymph basin can be performed. Metastases occur mainly as cutaneous lesions in the primary area and in the regional lymph basin, but can also develop in the lungs, peritoneum, retroperitoneum, bone, liver, breast, bladder, or ovary. (13) Complete remission using polychemotherapy can be found in the literature as early as 1987 in individual cases. (13,14) Recent individual reports of complete remission following chemotherapy using epidermal growth factor receptor-targeted therapy combined with radiotherapy as well as monotherapy using pembrolizumab show promising results. (15,16) Still, given the infrequency of porocarcinoma, and insufficient data on the treatment and outcomes, excision of benign poromas before malignant alteration in terms of prevention could be the most feasible approach to management.

CONCLUSION

Porocarcinoma still remains a relatively insufficiently explored tumor in the literature, as well as in clinical settings, with no firmly established official treatment protocols. Due to its similarity to squamous cell carcinoma, the verification of porocarcinoma in some cases requires analysis by a dermatopathologist, while the management of the tumor often requires a multidisciplinary approach. Since early diagnosis and treatment are of paramount importance, factors that cause a delay could be detrimental to a patient, and COVID-19 pandemic had a negative influence in different ways, as in our case.

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POROKARCINOM NOGE – DIJAGNOSTIČKI I TERAPIJSKI IZAZOVI I POTENCIJALNI UTICAJ PANDEMIJE KOVIDA 19 NA DIJAGNOZU I LEČENJE

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Sažetak

Uvod: Porokarcinom predstavlja retku, malignu, nemelanocitnu neoplazmu kože, porekla znojnih žlezda. Karakteriše ga visoka stopa metastaziranja, lokalne rekurentnosti i visok mortalitet. Definitivna dijagnoza može biti kompleksna zbog sličnosti sa skvamocelularnim karcinomom i Pedžetovom bolešću. Zvanični protokoli lečenja nisu utvrđeni, a hirurška ekscizija ostaje metoda izbora.

Prikaz slučaja: Pacijent starosti 76 godina, primljen je u našu kliniku u januaru 2020. godine zbog egzulcerisanog tumora desne natkolenice. U daljem toku je u više navrata hirurški lečen širokom lokalnom ekscizijom, uz ponovnu pojavu tumora na mestu operacije. Inicijalno je histopatološki dijagnostikovan slabo diferentovani skvamocelularni karcinom. Pacijent prestaje da dolazi na kontrole zbog oboljevanja i dugog oporavka od pneumonije izazvane Kovidom 19. Pri prvoj sledećoj kontroli u januaru 2022. godine dolazi do ponovne lokalne pojave tumora. Analizom od strane dermatopatologa postavljena je dijagnoza porokarcinoma. Nastavljeno je sa agresivnim lokalnim hirurškim lečenjem. U daljem toku lečenja, nakon EHO pregleda desnog ingvinuma postavlja se sumnja na metastatsku bolest, uz potvrdu zahvaćenosti limfnih čvorova biopsijom. CT grudnog koša i abdomena tada bez znakova diseminovane bolesti. Pri poslednjoj masivnoj lokalnoj pojavi tumora, MRI pregledom karlice prikazuje se sumnjiva masa u mokraćnoj bešici, radiološki konzistentna sa metastatskom bolesti, ali i mogućnošću drugog primarnog tumora. Pacijent je dalje upućen na onkološku i urološku evaluaciju.

Zaključak: Porokarcinom i dalje ostaje nedovoljno istražen tumor. Izostaju čvrsto utvrđeni, zvanični protokoli za lečenje. Zbog sličnosti sa skvamocelularnim karcinomom, verifikacija porokarcinoma može zahtevati analizu dermatopatologa, a tretman multidisciplinarni pristup. Rana dijagnoza i tretman su imperativ. Faktori koji odlažu takav pristup, poput pandemije Kovida 19 u prikazanom slučaju, mogu pogoršati prognozu i ishod.

Ključne reči: ekrini porokarcinom, porokarcinom, kožni tumori, Kovid 19

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ORIGINAL ARTICLE

Surgical treatment of solid variant of papillary thyroid carcinoma: fifteen-year experience of a tertiary center

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Summary

Aim: Papillary thyroid carcinoma (PTC) is a well differentiated, highly curable cancer, witha wide variety of histological forms. Although most of these variants are indolent, aggressive variants of PTC have been described. These variants include tall cell variant (TCV), hobnail variant (HV), columnar cell variant (CCV), diffuse sclerosing variant (DSV) and solid variant (SV). Solid variant represents one of the rarest forms of papillary thyroid carcinoma, with an incidence of about 2.6% according to retrospective studies.

Methods: In this retrospective study, data of all patients that underwent thyroid surgery in our Clinic between January 2008 and January 2018 were analyzed. All relevant information was obtained from a prospectively maintained institutional database.

Results: Of total of 1867 consecutive patients operated for follicular derived thyroid carcinomas during ten-year period, 38 had solid variant PTC as a definitive pathohistological finding (14 male and 24 female). The incidence of solid variant PTC is 2.04% in our series. Patient follow-up ranged from 36 to168 months, disese specific five-year survival rate was 97.4%.

Conclusion: Considering the scarcity of research discussing the prognosis of this variant of PTC, we believe that the treatment plan should be decided based on the extent of tumor and the experience of clinicians. WHO classification of thyroid neoplasms from 2022,may shed some light as to why the prognosis of solid variant PTC may be less aggressive than previously thought.

Keywords: thyroidectomy, papillary carcinoma, solid variant of papillary carcinoma

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INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common malignant endocrine tumor and represents up to 85-90% of all thyroid gland malignancies (1,2). PTC is well differentiated and has an indolent clinical course with a five-year survival rate of 97.5% and a ten-year survival rate of 93% (1,3). This refers to the classical form of PTC, while more aggressive forms are distinguished by higher degree of recurrence, local and vascular invasion, lymph node- and distal metastases. These aggressive forms are tall cell variant (TCV), hobnail variant (HV), columnar cell variant (CCV), diffuse sclerosing variant (DSV) and solid variant (SV) (1,3).

Solid variant represents one of the rarest variants of PTC, with an incidence of about 2.6% according to retrospective studies from the beginning of the century (4). Recent literature also confirms a similar incidence rate of about 3% (5).

The histological criteria for diagnosing solid variant of PTC are still a matter of debate, with a solid growth pattern necessary to include over 70% of the tumor mass, while according to some authors even 50% is sufficient to diagnose this variant (1). Further caution is advised in differentiating solid variantPTC and poorly differentiated thyroid carcinoma (PDTC). PDTC is distinguished by the presence of solid/trabecular/insulargrowth pattern, with the mandatory presence of two more criteria according to the Turin Consensus from 2017, namely the absence of a cytological features of the nucleus typical of papillary carcinoma and one of the following: convoluted nuclei, tumor necrosis and mitotic activity of ≥3 per 2mm^2 (6,7).

With the publishing of the fifth version of WHO histological classification of thyroid neoplasms in 2022, these histological differences are further elaborated, and as a result, an intermediate entity of differentiated high-grade thyroid carcinoma (DHGTC) is therefore introduced for PTC with ≥5 mitoses per 2 mm^2 and/or tumor necrosis, as well as pronounced invasive features to highlight high-risk differentiated thyroid carcinomas (8). The tumors classified as DHGTC may have retained cytological features typical of papillary carcinoma or a follicular or solid growth pattern.

The summary is that when it comes to classification of thyroid follicular neoplasms with solid growth patternwe can now differentiate between solid variant of PTC, differentiated high-grade thyroid carcinoma and poorly differentiated thyroid carcinoma that has the most aggressive histological features.

MATERIAL AND METHODS

Inthis retrospective study data of all patients that underwent surgical treatment for follicular derived thyroid carcinomas between January 2008 and January 2018 were analyzed. All relevant information was obtained from a prospectively maintained institutional database.

In all patients, neck ultrasound was performed preoperatively. Also, in all patients preoperative FT4, TSH, thyroglobulin (TG) and TPO-antibody levels were determined before surgery. FNAB was performed in most patients.

We have used the following criteria for the diagnosis of solid variant PTC: more than 70% of solid growth pattern of the primary tumor, nuclei appearance that matches the appearance of the nuclei of classic variant papillary carcinoma and the absence of tumor necrosis.

The results are shown as tables and graphs. Depending on the type of variables and the normality of the distribution, the data description is shown as n (%), arithmetic mean ± standard deviation. In survival rate analysis, the Kaplan-Meier method was used. T-test was used for testing statistical hypothesis. All statistical tests received the same level of significance with a p value of <0.05. All data was processed in IBM SPSS Statistics version 25 (SPSS Inc., Chicago, IL, USA) software package.

RESULTS

Of total 1867 patients operated for follicular derived thyroid carcinomas during ten-year period, 38 had solid variant of papillary carcinoma. Among them, 14 were males and 24 females with an average age of 54.7±2.4 (from 20 to 79). The incidence of solid variant in our series was 2.04%.

Patients were admitted with different admission diagnoses, withtwopatients having the preoperative diagnosis of Mb.Graves, and twelve patients with a suspected thyroid tumor, based on the findings of a fine needle aspiration biopsy (FNAB) or on the basis of the preoperative physical findings. The distribution of patients based on the admission diagnosis is presented in **Table 1**.

Table 1. The distribution of patients based on the referral diagnosis

Admission diagnoses	Number	Percent (%)
Thyroid tumor	5	13.2
Solitary nodule	13	34.2
Solitary nodule (suspected of tumor)	3	7.9
Recurrent goitre	1	2.6
Multinodular goitre	9	23.7
Multinodular goitre (suspected of tumor)	4	10.5
Toxic adenoma	1	2.6
Mb. Graves	2	5.3
Total	**38**	**100**

Fine needle aspiration biopsy was performed on 26 patients. The distribution of patients according to FNAB findings with matching cytological diagnosis based on The Bethesda System for Reporting Cervical Cytology is presented in **Table 2**. In 16 patients, the FNAB indicated benign thyroid conditions. Eight patients were suspected

to have a follicular neoplasm, and one patient was suspected to have an oncocyticneoplasm. According to the FNAB findings papillary thyroid cancer was highlysuspected in only two patients. In eleven patients with later confirmed pathohistological diagnosis of SV PTC, fine needle aspiration FNAB was not performed, among them two patients with Graves' disease and one patientwith toxic adenoma.

Table 2. The distribution of patients according to FNAB findings

Finding	Number	Percent (%)
FNAB not performed	11	31.6
Benign – Bethesda II	14	34.2
Hashimoto thyroiditis – Bethesda II	2	5.3
Follicular lesion – Bethesda IV	8	21.1
Oncocytic lesion – Bethesda IV	1	5.3
Papillary thyroid carcinoma – Bethesda V/VI	2	2.6
Total	**38**	**100**

Total thyroidectomy was performed in 27 patients, thyroid lobectomy in 8 patients, near total thyroidectomy in one patient and in one patient with preoperatively enlarged lymph nodes total thyroidectomy and neck dissection were performed **(Table 3)**.

Table 3. Type of surgical procedure performed

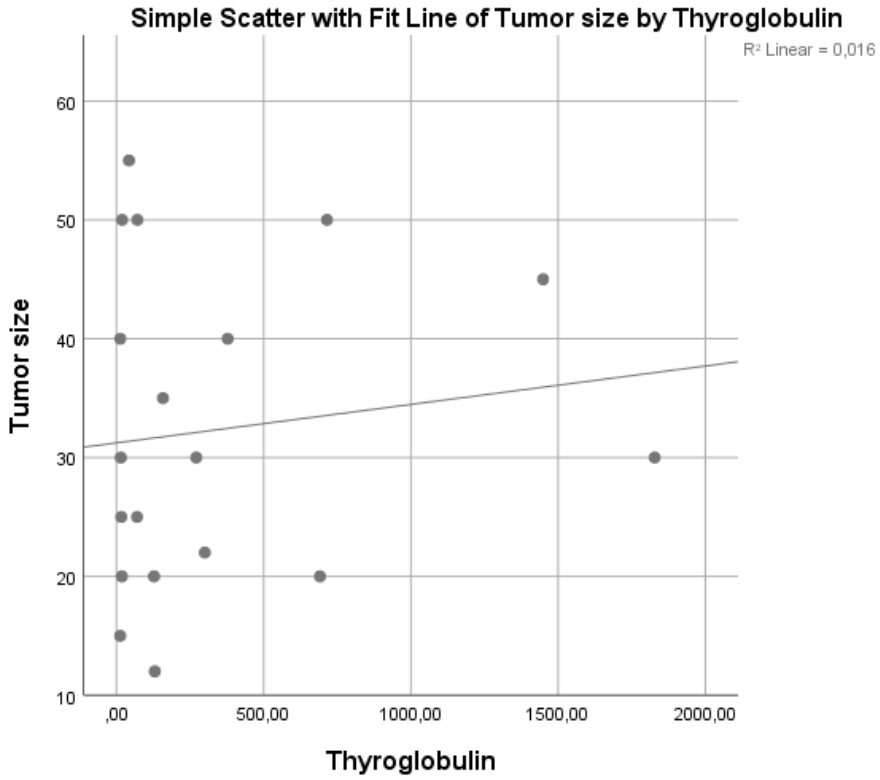
Procedure	Number	Percent (%)
Total thyroidectomy	27	71.1
Lobectomy	7	18.4
Near-total thyroidectomy	3	7.9
Thyroidectomy with neck dissection	1	2.6
Total	**38**	**100**

Three patients had slightlyelevated basal calcitonin levels preoperatively (between 12 and 13.5 ng/L). Preoperative thyroglobulin values in operated patients ranged from 12.8 to 1828.0 pg/mL, average of 332.9 ± 117.6pg/mL. Statistical analysis showed that there is no statistically significant correlation between the size of the tumor and the preoperative value of thyroglobulin (p = 0.61) **(Fig. 1)**.

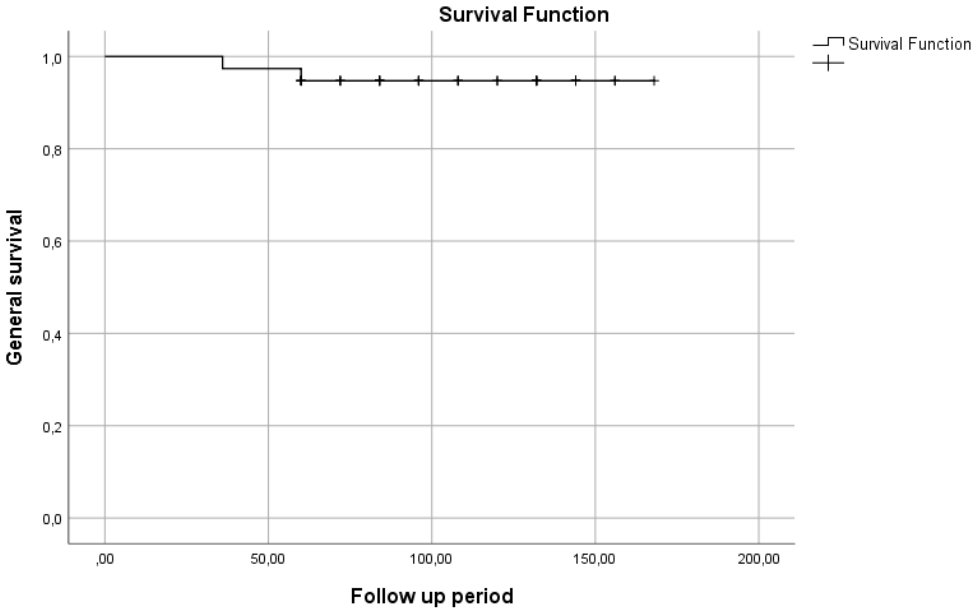
The tumor size ranged from 12 to 60 mm, with an average of 31.24±2.36 mm, of that 63.2% being > 2 cm in size.Extrathyroidal extension was found in two patients, vascular invasion in one patient, and both vascular invasion and extrathyroidal extension was found in one patient who had undergone thyroidectomy with neck dissection. Twenty-four patients (63.2%) havebeen treated with radioiodine therapypostoperatively.

All patients had their thyroglobulin values measured postoperatively, except for the lobectomy patients. All patients had values of thyroglobulin lower than 0.2 pg/mL, except for one female patient, aged 41, who had postoperative thyroglobulin level of 5.4 pg/mL after near total thyroidectomy. The patient was reoperated, and then subsequently referred to radioiodine therapy, after which the control thyroglobulin level was <0.2 pg/mL.

At the end of the follow up, one patient died as consequence of malignancy, three years after surgery, and one patient died of an unrelated condition (acute mesenteric ischemia), five years after surgery. This makes overall five-year survival of 94.7% **(Fig.2)**, and disese specific five-year survival of 97.4% **(Fig.3)**. The meanfollow-up periodwas103.9±5.5 months (36-168 months), or 8.6 years.



Graph 1. Correlation between the tumor size and preoperative thyroglobulin values



Graph 2. Overall survival (Kaplan-Meier)

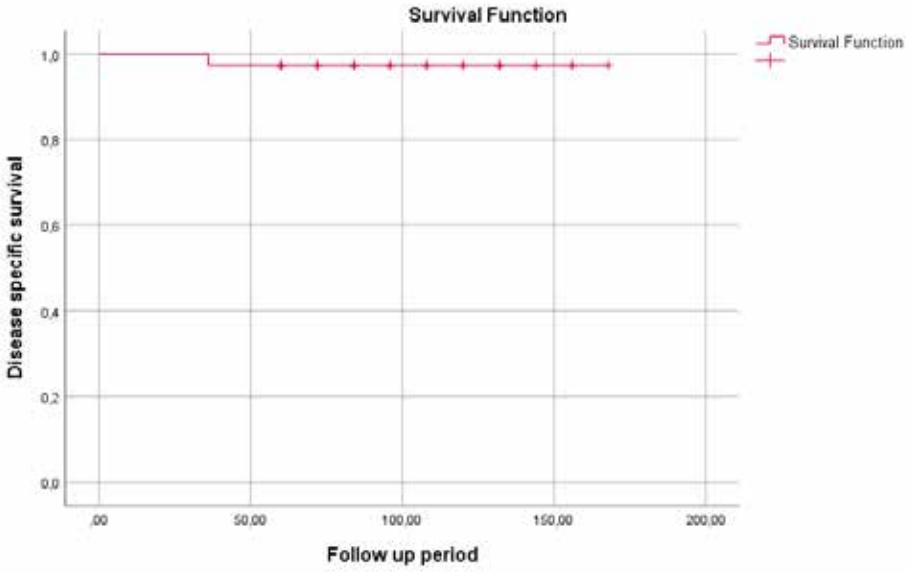
DISCUSSION

Solid variant of PTC was particularly associated with ionizing radiation as a risk factor. Data supporting this evidence is the presence of SV in 37% of all PTCs after the Chernobyl nuclear disaster (6). However, in a recent publication by Chang et al., none of 14 patients with solid variant PTC had ionizing radiation as an established risk factor (9).

Solid variant of PTC was previously associated with younger age, but now it is known that it most often occurs in the fifth decade of life, predominantly in women (9,10). In our study most patients were female, with the average age of 54.7 years. Research by Ohashi et al. (13) shows the similar average age of the patients -59.1 years. In the study by Nikiforov et al., the data of 20 patients operated for the solid variant PTC were analyzed (4). The male to female ratio was 1:3 (with the average age of 35.5), while in our study this ratio was 1:1.7.

Solid variant as a subtype of PTC has not been sufficiently studied due to its low incidence and its biological behavior is still thought to be uncertain.Authors frequently stated that the presence of a solid component does not affect the prognosis (6). After publication of WHO's new classification guidelines this is mostlytruthful if other histological features don't place a thyroid tumor with asolid growth pattern within DHGTC or PDTC.

Five-year overallsurvival in our study was 94.7%, with disease specific survival being 97.4%, and the average follow up period of 103.9 months (8.6 years), while Nikiforov et al. studyshows disease-specific survival of 90% after an average follow-up of 18.7 years(4). Patients with



Graph 3. Disease specific survival curve (Kaplan-Meier)

solid form of PTC have a better survival compared to patients with PDTCand whose survival according to the study by Carcangiu et al. is 44% after an average follow-up of 3.7 years (14).

The main clinical characteristics of SV PTC are, according to a meta-analysis that included 11 studies with a total of 205 confirmed solid variant PTC cases, an increased risk of vascular invasion, local tumor recurrence and increased mortality compared to the classical form of PTC. Statistically significanthigher risk of lymph node and distal metastases compared to the classical form was not found (2). In contrast, some authors attribute the aggressiveness of solid variant PTC primarily to the increased frequency of lymph node and distal metastases, as well as the occurrence of local recurrences (9). In his study, Nikiforov compares patients with solid variantPTC with patients who have a classic form of PTC and concludes that patients with solid growth tumors have the same rate of local recurrences, but a higher rate of distant metastases(4). In our study, two patients have had extrathyroidal invasion, one patient had vascular invasion, and one patient was found to have both extrathyroidal and vascular invasion with lymph node metastases, and in that patient both thyroidectomy and neck dissection were performed. Only one patient in have had local recurrence.

In addition to the above-mentioned characteristics, Ohashi et al. (13) also mention a larger tumor size of solid subtype of PTC(mean size being 28 mm) compared to the classical form PTC. In contrast, Chang et al. showed an average tumor size of 1.02 cm in the sample of 14 patients, with no patients having tumors over 2 cm in diameter (9). Meta-analysis by Vuong et al. found no statistically significant difference in tumor size between these two forms (60.4% of SV PTC and 53.8% of classical PTC being over 2 cm in diameter) (2). Our study shows the average size of solid variant PTC of 31 mmof that 63.2% being over 2 cm.

In our study preoperative thyroglobulin value had no statistically significant correlation to tumor size. This is in contrast with a study by Patteli et al. that investigated whether TG value positively correlated with thyroid gland size (r2 0.49, $p < 0.001$), as well as malignant tumor size (r2 0.27, $p < 0.001$) in a large sample of 422 patients (15). The difference in the results could be attributed to the relatively low cut-off value in some of the laboratories (around 300 pg/mL). Many authors have certainly pointed out the unreliability of TG as a tumor marker, that is, the inadequate use of TG as a predictor for the malignant potential of a nodule. It was Patelli and colleagues who obtained a very low sensitivity and a low positive predictive value in the use of TG for these purposes (Sn 10.3%, PPV 21.4%, for a cut-off of 500 ng/L). These conclusions are vastly present in everyday practice, and the stance that TG is not to be used as a tumor marker has been largely adopted, except in thyroidectomy patients.

In our study, three patients had preoperative basal calcitoninslightly elevated (12.1, 12.8 and 13.5 ng/L), most likely because of reactive C-cell hyperplasia, which was shown to be a common incidental finding in patients with follicular cell tumors (16).

CONCLUSION

The aim of this study was to evaluate and present the experience of our clinic with the surgical treatment of solid variant PTC. The experience with tumors who express solid growth is still scarce. These tumors have been mostly described as highly aggressive, especially when compared to classic variant of PTC, with high rate of recurrence, extrathyroidal and vascular invasion, and even high lethality, especially in older literature.

When it comes to SV PTC our research shows a very low rate of recurrence, lymph node metastasis and lethality, leaving 5-year survival rate well over 90%, and the most recent WHO classification of thyroid neoplasms shines light as to why this might be so. Much of the previous work done on this topic didn't have as clear criteria on what should be classified as SV PTC, classifying more aggressive forms (now established as DHGTC and possibly even PDTC in some cases) as solid form of PTC.

All cases in our study were classified as SV PTC and no pathohistologicalfindings fulfilled the criteria to be classified in DHGTC or PDTC.The new classification may break down some contradiction found in literature on this topic.

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HIRURŠKO LEČENJE SOLIDNE VARIJANTE PAPILARNOG KARCINOMA ŠTITASTE ŽLEZDE: PETNAESTOGODIŠNJE ISKUSTVO TERCIJARNOG CENTRA

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Sažetak

Cilj: Papilarni karcinom štitaste žlezde je dobro diferenovani karcinom sa brojnim histološkim tipovima. Iako je većina histoloških tipova papilarnog carcinoma veoma indolentna, opisuju se i agresivnije histološke varijante u koje spadaju vatrijanta sa visokim ćelijama, varijanta sa kolumnarnim ćelijama, difuzno sklerozantna varijanta i solidna varijanta. Solidna forma papilarnog carcinoma predstavlja jednu od najređih histoloških varijanti sa incidencijom od oko 2,6% prema retrospektivnim studijama.

Metode: U ovoj retrospektivnoj studiji analizirani su podaci svih operisanih pacijenta u period između januara 2008. I januara 2018. godine. Sve relevantne informacije dobijene su iz prospektivno vođene baze podataka koja je implementirana u svakodnevi rad naše klinike.

Rezultati: Tokom posmatranog vremenskog perioda, zbog malignih tumora štitaste žlezde porekla folikularnih ćelija operisano je 1867 pacijenata. Od toga, 38 pacijenta je imalo solidnu varijantu papilarnog carcinoma štitaste žlezde. Incidencija solidne varijante papilarnog carcinoma stoga iznosi 2,04% u našoj studiji. Period praćenja se kretao od 36 do 168 meseci, a petogodišnje preživljavanje 97,4%.

Zaključak: Obzirom na mali broj radova koji se bavi prognozom solidne varijante papilarnog karcinoma, verujemo da plan lečenja treba da bude zasnovan na iskustvu kliničara i veličini tumora. Poslednja klasifikacija tiroidnih neoplazmi Svetske zdravstvene organizacije iz 2022. godine može doprineti razumevanju zašto je solidna varijanta papilarnog carcinoma manje agresivna nego što je ranije smatrano.

Ključne reči: tiroidektomija, papilarni karcinom, solidna varijanta papilarnog karcinoma

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