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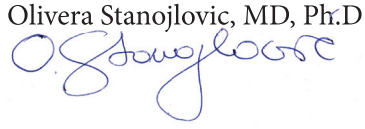

CONTENTS

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ORIGINAL ARTICLE

Expression of major hemoglobin haplotypes in the first twenty-four months of life suggests a gradual decline of normal hemoglobin A among infants of African descent

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Summary

Introduction: Hemoglobin is the principal protein in red blood cells and is responsible for delivering oxygen from the lungs to other body parts. Understanding the hemoglobin type profile of infants and the patterns of expression in the first twenty-four months of life is a significant area of research that can provide crucial insights into infant health and development.

Material and Methods: The study population consisted of 147 infants (male and female) aged 9 to 24 months. Participants were recruited from the pediatric and sickle cell clinics and the medical laboratory department of Rivers State University Teaching Hospital (RSUTH) in Port Harcourt, Nigeria. The hemoglobin type was determined using high-performance liquid chromatography (HPLC) (D-10, Bio-Rad).

Results: The median (range) values of the hemoglobin types were: HbA 70% (22-98), HbF 10% (0-50), HbS 0% (0-78)and A2 CE 0% (0-50). Hemoglobin A expression was 65% at nine months, 79% at 12.5 months, 46% at 22 and 60% at 24 months. HbF expression was 21% at nine months, 10% at 12 months, 24% at 15.5 months, 0.25% at 21 months, and 12% at 24 months. HbS was 0.8% at nine months and 0% at 16 months. 50% at 22 months and lastly 22% at 24 months. The HbA2 was 0.5% at nine months and 12% at 11 months, 0% at 21 months and lastly 0.2% at 24 months. HbA, HbF, and HbA2 were negatively correlated with age, while HbS was positively correlated with age.

Conclusion: The pattern of expression of the four hemoglobin types in this study was age-dependent. Sex was not found to influence the expression of hemoglobin types in infants. There is a gradual reduction in the expression of normal hemoglobin A and a gradual increase in abnormal hemoglobin S among infants of African descent.

Keywords: hemoglobin types, percentage expression, sickle cell disease, beta thalassaemia, HbA, HbF, HbS, HbA2

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INTRODUCTION

Hemoglobin, the principal protein in red blood cells, carries oxygen in the blood. In infancy, the natural replacement of fetal hemoglobin (HbF) by adult hemoglobin (HbA) is a normal developmental process. HbF, comprising two alpha-globulin and two gamma-globulin chains ($\alpha_2\gamma_2$), is the major hemoglobin in fetal red blood cells during gestation, constituting 60 to 80 percent of total hemoglobin in full-term newborns. Around 6 to 12 months of age, HbF is gradually and naturally replaced by adult hemoglobin (HbA; $\alpha_2\beta_2$). This natural transition is a part of an infant's development, as HbA becomes the predominant type, gradually replacing HbF (1,2).

At Birth, HbF (fetal hemoglobin) accounts for approximately 80% of total hemoglobin while HbA (adult hemoglobin) accounts for 20%. The transition from gamma-globin synthesis (HbF) to beta-globin synthesis (HbA) begins before birth and continues in the first 24 months of life. By approximately six months, healthy infants will have mostly HbA, a small amount of HbA2, and negligible HbF. Therefore, HbA2 levels in infancy are typically as follows:0-30 days: 0.0-2.1%, 1-2 months: 0.0-2.6%, 3-5 months: 1.3-3.1%,> 6 months: 2.0-3.3%2 (3).

Hemoglobin S (HbS), an abnormal variant associated with sickle cell disease, is primarily expressed in infancy but its levels are typically low. If one parent carries the sickle cell trait (HbAS) and the other has normal hemoglobin (HbAA), the child will have HbAS (sickle cell trait). HbAS (sickle cell trait) is characterized by one normal beta-globin gene (HbA) and one sickle beta-globin gene (HbS). At six months, the levels of HbS decrease significantly, and over six months, the HbS level remains at a low percentage (3,4).

Studying the expression and pattern of development of hemoglobin types in people of African descent is crucial for several reasons, namely:

a) Sickle cell disease (SCD) is prevalent among people of African descent. Over 75% of the approximately 300,000 children born with sickle cell anemia each year are in sub-Saharan Africa (5). The mutation responsible for sickle cell disease originated in Africa and persists due to its protective effect against severe Plasmodium falciparum malaria. In some sub-Saharan African areas, up to 2% of all children are born with this condition (6). Understanding hemoglobin variants, such as HbS (sickle cell hemoglobin), helps diagnose and manage SCD effectively.
b) Thalassemia is an inherited blood disorder affecting hemoglobin production. Some thalassemia variants are more common in African populations (3). Studying these variants aids in early detection and appropriate management.
c) African populations exibit high genetic diversity. Investigating hemoglobin variants contributes to our understanding of human evolution and adaptation.
d. Accurate diagnosis and treatment depend on recognizing specific hemoglobinopathies. Research informs guidelines for prenatal screening, genetic counseling, and disease prevention;
e. In medical laboratories, understanding hemoglobin types ensures accurate results. Quality control measures prevent misdiagnoses and improve patient care.

In infants, the interaction between hemoglobins HbA, HbF, HbS, and HbA2 plays a crucial role in understanding various hemoglobinopathies and disorders. HbF, the predominant fetal hemoglobin, declines after birth, while HbA becomes the primary hemoglobin in adults, along with HbA2 and trace amounts of HbF (7). Hemoglobin variants like HbS can affect the determination of HbA2 levels, leading to biases in measurement (8). The precise measurement of HbA2 is crucial for diagnosing beta thalassemia trait, but other hemoglobin variants like HbS, HbC, HbE, or HbD can complicate the interpretation of results (9,10). Understanding the intricate balance and interactions between these hemoglobin types is essential for diagnosing and managing infant hemoglobin disorders.

Most of the values encountered in the literature concerning the pattern of expression of these hemoglobin types are often Caucasian values with minimal reference to people in the sub-Saharan Africa. This study was aimed at detecting various hemoglobin types and studying the patterns of expression of these hemoglobin types in the first twenty-four months of the life of infants of African descent.

MATERIAL AND METHODS

Study Area

This study was conducted at Rivers State University Teaching Hospital in Port Harcourt, Rivers State, Nigeria. The geographical coordinates of Rivers State are approximately latitude 4.7497 and longitude 6.8277. The hospital, formerly known as Braithwaite Memorial Specialist Hospital (BMSH), is a government-owned facility in Old GRA, Port Harcourt, Rivers State, Nigeria. The hospital serves as a state-of-the-art teaching facility for medical students and other health professionals from Rivers State University. With the capacity of 375 beds, RSUTH houses various departments, including Medicine, Pediatrics, Laboratories, Radiology, Family Medicine, Obstetrics & Gynecology Anesthesia, Surgery, Chemical Pathology, Hematology & Blood Transfusion, Medical Microbiology, Anatomical Pathology, Ophthalmology, and Accident and Emergency.

SAMPLE SIZE CALCULATION

The minimum sample size was calculated using the Cochran standard formula as described by Patra (11).

$$n = \frac{Z^2Pq}{d^2}$$

Where,

n= minimum sample size required

Z= Standard normal deviation, set at 1.96, corresponding to a 95% confidence level

P= Proportion of sickle cell disease patients = 10% = 0.1

q= 1-P = 1-0.1 = 0.9

d = Level of precision = 0.05

Applying this formula,

$$n= \frac{1.96^2 \times 0.1 \times 0.9}{0.05^2} = 138.3$$

Therefore, the minimum sample size for this study = 139.

STUDY POPULATION

The study population consisted of 147 infants (male and female) aged 6 to 24 months. Participants were recruited from the pediatric and sickle cell clinics and the laboratory department of Rivers State University Teaching Hospital in Port Harcourt, Rivers State, Nigeria.

Ethical Approval; This study received ethical approval from the Rivers State University Teaching Hospital (RSUTH) ethics and research committee, Port Harcourt, Nigeria.

Informed Consent: The participants' parents voluntarily signed written informed consent forms in their handwriting as proof of their willingness to provide samples for the tests.

Collection of Sample: Two milliliters of whole blood were collected using an S-monovette vacutainer syringe. The blood was deposited into an anticoagulant containing ethylenediaminetetraacetic acid (EDTA) and used for the HPLC analysis.

Study Design. The study was cross-sectional.

Procedure

The hemoglobin type was determined using high-performance liquid chromatography (HPLC) (D-10 instrument;Bio-Rad).

PRINCIPLE

In this method phosphate buffers at different concentrations (mobile phase), pass under pressure through an ionic exchange column (stationary phase). The stationary phase consists of a temperature controlled analytical cartridge containing a resin of anionic or cationic particles (3-5 µm). The chromatographic station delivers a programmed buffer gradient of increasing ionic strength and pH to the cartridge by two dual-piston pumps, and the hemoglobin variants are separated according to their ionic interaction with the stationary phase.

The separated hemoglobin then pass through the flow cell of the filter photometer, where changes in the absorbance (415 nm) are measured; background variations are corrected by an additional filter at 690 nm. Each hemoglobin is characterized by a specific retention time, the elapsed time from the sample injection to the apex of a hemoglobin peak.

The calibration factors for HbA2, F, A1C are automatically calculated by processing a calibration sample at the beginning of each run. Specific software turns the raw data collected from each analysis into a report showing the chromatogram, with all the hemoglobin fractions eluted, the retention times, the areas of the peaks and the values (%) of the different hemoglobin components. The report presents the percentages of hemoglobin types F, A1C, A and A2 and provides qualitative and quantitative determination of abnormal hemoglobin types.

Procedures were followed as contained in the standard operating procedures.

STATISTICAL ANALYSIS

A structured approach was adopted for statistical analysis, beginning with an initial assessment of the distribution of hemoglobin variants (HbA, HbF, HbS, HbA2), including evaluations of normality and variability. Subsequently, the median and range for each measure were determined overall and stratified by sex (female vs. male) and age group (0–9, 10–19, 20–29 years). Box plots were used to illustrate the distribution of data points across the variables. For parameters that did not follow a normal distribution, the Mann–Whitney U test was used to compare two groups (sex), while the Kruskal–Wallis test was applied for comparisons involving more than two groups (age group). Spearman's correlation analysis was performed to assess the strength and direction of relationships between hemoglobin variants, as represented by the correlation coefficient (rho). All statistical tests were two-tailed, with a significance threshold set at $p < 0.05$. Data management, statistical analysis, and visualizations were conducted using SAS JMP Statistical Discovery Software (version 16.2; SAS Institute Inc., Cary, NC, USA).

RESULTS

Table 1 presents the key findings on the median (range) value of hemoglobin parameters of 147 participants, categorized by sex and age groups. The median (range) values were: HbA 70% (22-98), HbF 10% (0-50), HbS 0% (0-78) and A2 CE 0% (0-50). The p-value for hemoglobin A

Table 1. Comparison of Mean Parameters of hemoglobin variants by demographics

Characteristic	n	HbA (96.8-97.8)	HbF (0.8-2.0)	HbS (0.0-0.5)	A2 CE (2.2-3.2)
		median (range)	median (range)	median (range)	median (range)
Overall	147	70 (22-98)	10 (0-50)	0 (0-78)	0 (0-50)
Sex					
Female	77	71 (30-95)	10 (0-50)	0 (0-61)	0 (0-50)
Male	70	67 (22-98)	10 (0-50)	0 (0-78)	0 (0-30)
p-value		**0.041**	0.795	0.063	0.070
Age group (months)					
0-9	21	70 (30-92)[a]	21 (6-50)[a]	0 (0-45)[a]	0 (0-20)[a]
10-19	85	75 (39-98)[a]	10 (0-50)[a]	0 (0-61)[a]	0 (0-50)[a]
20-29	41	51 (22-91)[b]	1 (0-42)[b]	42 (0-78)[b]	0 (0-8)[ab]
p-value		**<0.001**	**<0.001**	**<0.001**	**0.001**

p-values in bold *are statistically significant at the level of probability indicated.*

type in relation to sex was 0.041. No statistically significant differences were observed with other hemoglobin types in relation to sex. Age was found to exert significant differences in all the hemoglobin types with a p-value of less than 0.001.

Table 2 shows the Spearman's pairwise correlation Analysis of the hemoglobin variants. A significant negative correlation exists between HbA and Age (rho=-0.37, p<0.001), HbF and Age (rho=-0.32, p=0.001), and a significant positive correlation between HbS and Age (rho=0.49, p<0.001). A significant negative correlation also existed between HbA2 and age (rho=-0.34, p<0.001). There is a negative correlation between HbS and HbA2 (rho=-0.29, p<0.001), as well as between HbS and HbF (rho=-0.58, p<0.001). HbS is the only hemoglobin type that showed positive correlation with age (rho=0.49, p<0.001)

Table 3 shows the pairwise correlation analysis of the hemoglobin variants by Age Groups. HbF vs. HbA shows a negative correlation in the 0-9 months group (rho=-0.45, p=0.040) and 10-19 months (rho=-0.46, p<0.001). No significant correlation was found in the 20-29-month groups. HbS vs. HbA exhibits a negative correlation in groups 0-9- and 20-29-months respectively (-0.61, p=0.004) and (-0.86, p<0.001). HbS vs. HbF shows no significant correlation in the 0-9 months group but exhibits a negative correlation in the 10-19- and 20-29-month groups, (rho=-0.43, p<0.001 and rho=-0.56, p<0.001 respectively). A2 CE vs. HbF has a positive correlation in the 20-29 months group rho=0.36, p=0.019 and negative correlations in the 0-9- and 10-19-months group (rho=-0.46, p=0.035 and rho=-0.37, p<0.001 respectively).

Box Plots presents the parameters' distribution by age and sex, highlighting variations and trends as shown in **Figures 1-3.**

Figure 1 shows the trend in the development of hemoglobin types according to the age of the infants. There was a gradual rise in the percentage rise in the hemoglobin A type which peaked at 79% by the 12.5 month. After that, there was a decline in the HbA, up to 46%, at 22 months, which was the lowest point. From this point, a steady rise in the percentage of HbA further increased and peaked at 60% at 24 months.

For HbF, at nine months, it was 21%. This value dropped to 10% at 12 months, then assumed a steady increase and peaked at 24% by 15.5 months. Another drop was observed, which settled at 0.25% by 21 months. The value resumed another rise and peaked at 12% by 24 months.

HbS at nine months was 0.8%, which dropped slightly and settled at 0% at 16 months. After that, there was a steady increase, which peaked at 50% by 22 months and later drastically rose to 22% at 24 months.

The HbA2 value at 9 months was 0.5%. This value increased and peaked at 12% by 11 months. After that, it gradually fell to 0% by 21 months and increased steadily to 0.2% by 24 months.

Table 2. Pairwise Correlation Analysis of the hemoglobin variants

Spearman's correlations		Age (months)	HbA (%)	HbF (%)	HbS (%)
HbA (%)	rho	-0.37			
	p-value	**<0.001**			
HbF (%)	rho	-0.32	-0.03		
	p-value	**<0.001**	0.728		
HbS (%)	rho	0.49	-0.63	-0.58	
	p-value	**<0.001**	**<0.001**	**<0.001**	
A2 CE (%)	rho	-0.34	0.19	-0.12	-0.29
	p-value	**<0.001**	**0.022**	0.164	**<0.001**

rho - Spearman's correlation coefficient
p-values in bold *are statistically significant at the level of probability indicated*

Table 3. Pairwise Correlation Analysis of the hemoglobin variants by Age Group

Variable	by Variable	0-9 (months)		10-19 (months)		20-29 (months)	
		rho	p-value	rho	p-value	rho	p-value
HbA (%)	HbF (%)	-0.45	**0.040**	-0.46	**<0.001**	0.17	0.295
HbA (%)	HbS (%)	-0.61	**0.004**	-0.17	0.123	-0.86	**<0.001**
HbA (%)	A2 CE (%)	0.41	0.068	-0.13	0.253	0.12	0.449
HbF (%)	HbS (%)	-0.21	0.351	-0.43	**<0.001**	-0.56	**<0.001**
HbF (%)	A2 CE (%)	-0.46	**0.035**	-0.37	**<0.001**	0.36	**0.019**
HbS (%)	A2 CE (%)	-0.31	0.167	0.01	0.911	-0.29	0.062

p-values in bold *are statistically significant at the level of probability indicated*

Figure 2 shows a Box Plot showing trends of hemoglobin types development by Age and Sex. The pattern of expression was similar in both male and female participants.

Box Plot of hemoglobin variants showing trends by Age Group and Sex is shown in **Figure 3**. The trend of expression and development is similar in both groups

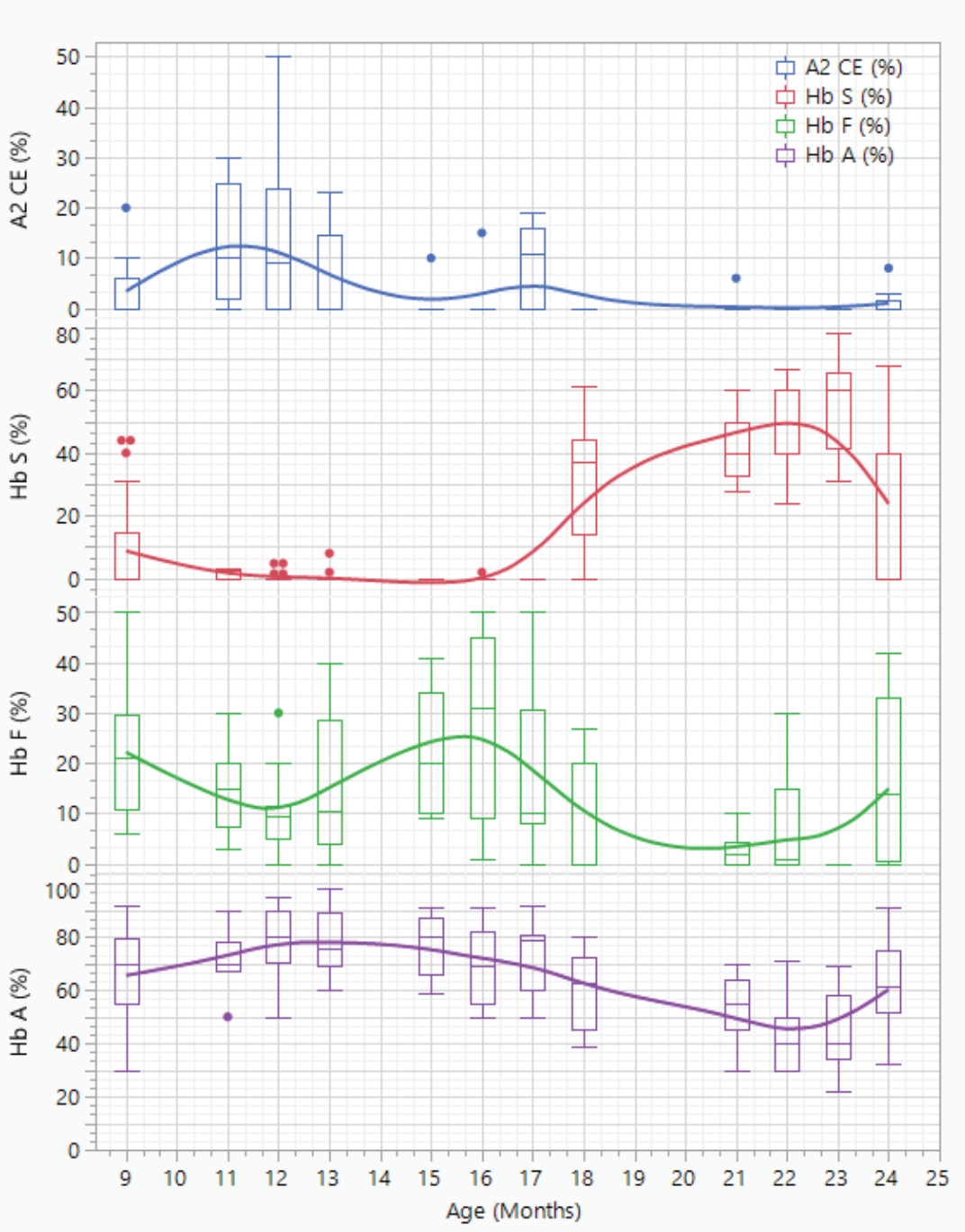

Figure 1. Box Plot of hemoglobin variants showing the trend of development by Age

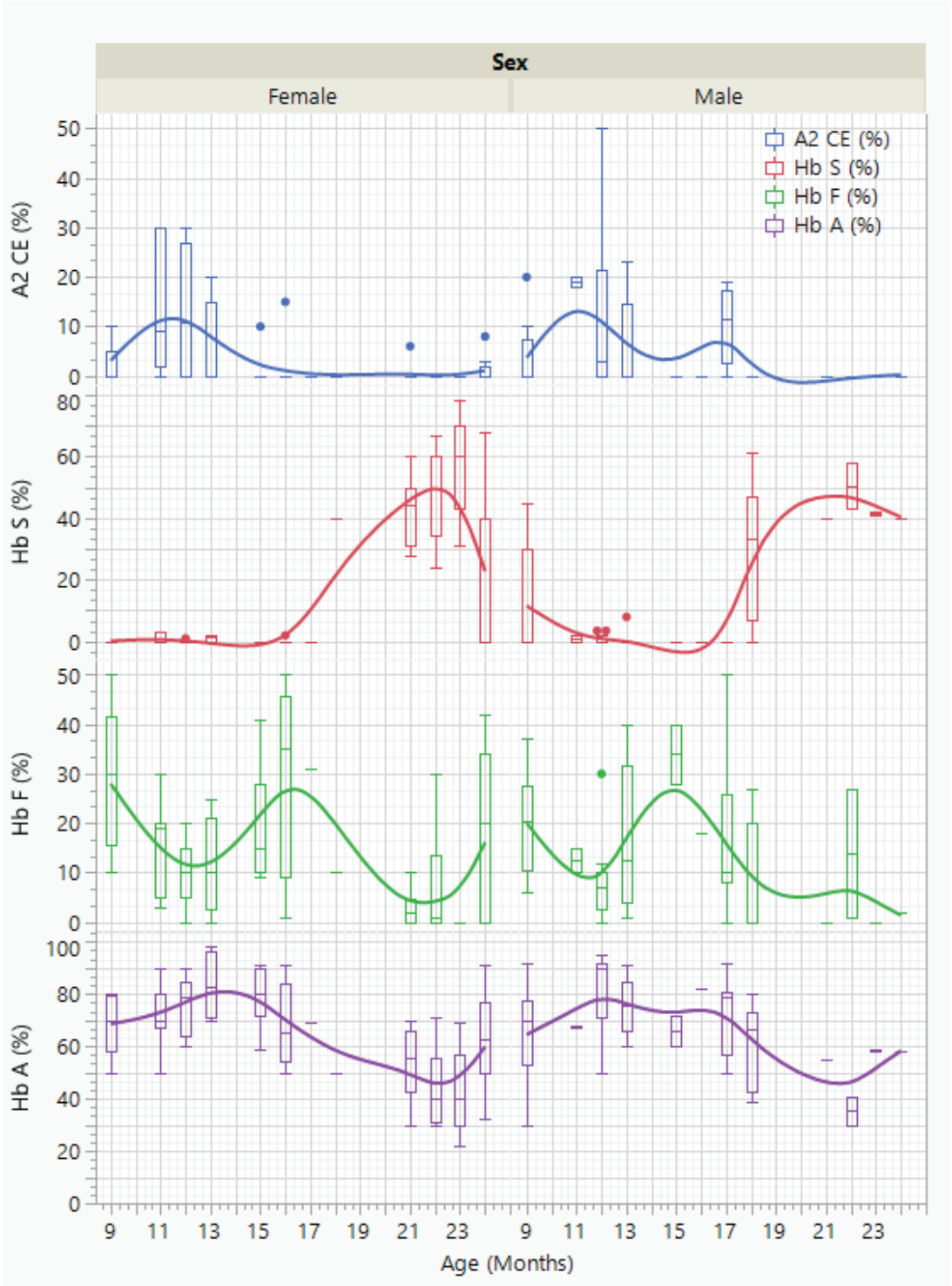

Figure 2. Box Plot showing trends of hemoglobin development by Age and Sex

DISCUSSION

This study aimed to detect the major hemoglobin types and investigate the pattern of expressions of these napisati hemoglobins in infants during their developmental ages of nine to twenty-four months. The key findings of the study are as follows: the average percentage expressions of HbA, HbF, HbS, and HbA2 were 70% (22-98), 10% (0-50), 0% (0-78) and 0% (0-50) respectively. The result shows normal HbA levels, which signify normal hemoglobin switching and erythropoiesis maturation. In cord blood, HbA levels are 21.14±7.04% and increase to 83.38±1.31% in the sixth month. In contrast, HbF levels are 78.39±7.59% and rapidly decrease in the first six months, according to Wong et al. (12). This is consistent with our findings that HbA levels are predominant in this age group, reflecting the transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA).

Elghetany and Banki (1) had earlier reported that, from birth to adulthood, there are notable shifts in the relative amounts of different hemoglobin fractions, such as HbA, HbF, and HbA2. Specifically; HbF, the predom-

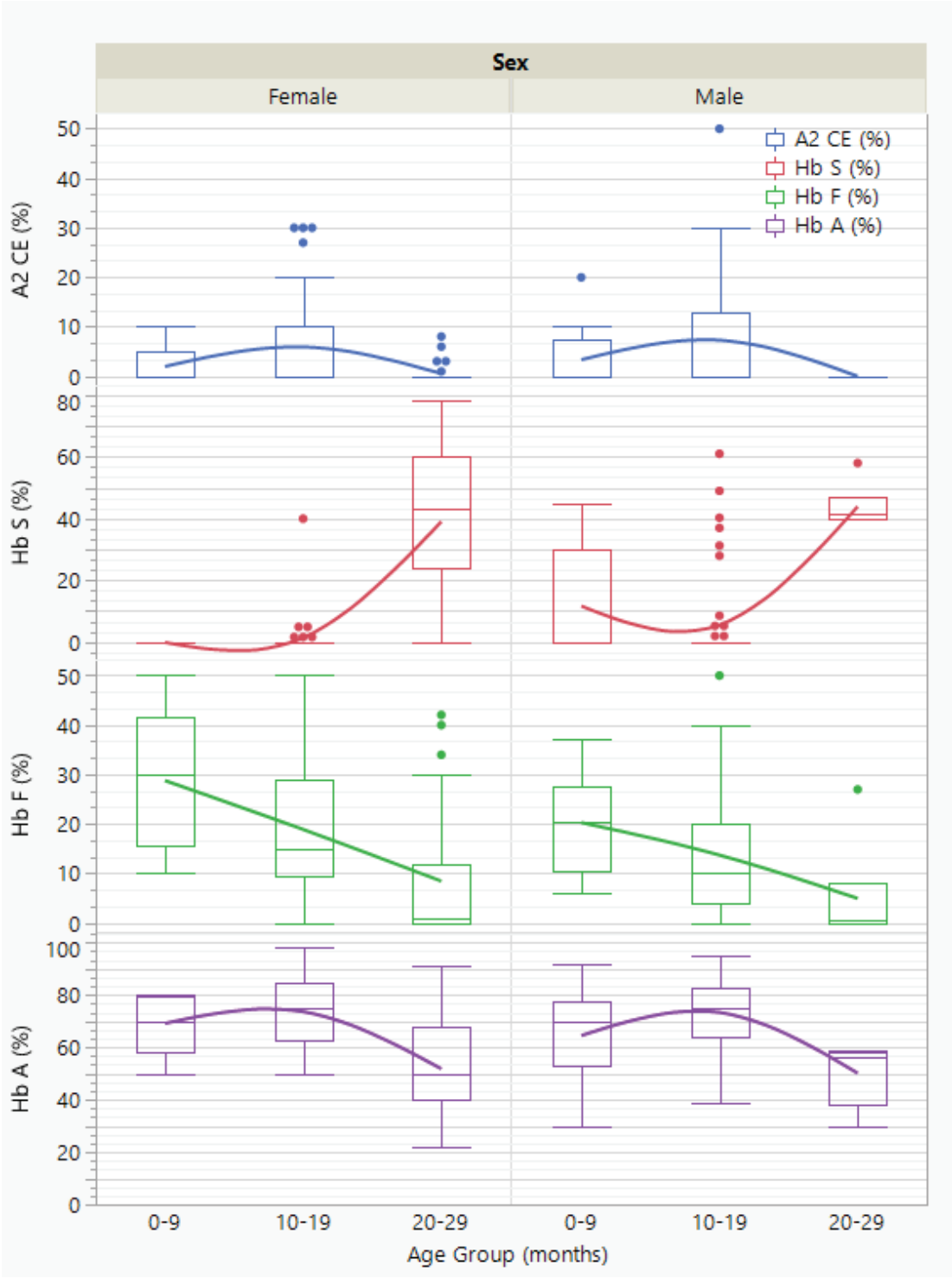

Figure 3. Box Plot of hemoglobin variants showing trends by Age Group and Sex

inant hemoglobin in fetal life, gradually decreases from 50-80% at birth to 1-2% by six months of age and beyond. HbA, the primary adult hemoglobin, increases from low levels at birth to 95-98% by adulthood. HbA2, a minor adult hemoglobin, increases from low levels at birth to 2-3% by adulthood.

The notable shifts in hemoglobin types with age are attributed to the regulation of globin gene expression during the developmental stage of erythropoiesis from fetal to adult. This process, as detailed in references (13,14), plays a crucial role in the transition from fetal to adult hemoglobin types.

HbS levels were observed to increase significantly with age, with the highest levels in the 20–29-month group, which also aligns with a study (4,16). The increased HbS levels with age may be linked to the genetic expression of sickle cell traits becoming more pronounced over time. This finding reiterates the importance of early detection for managing HbS, and underscores the potential for this research to contribute to improved management strategies for sickle cell disease.

It was observed in this study that females had elevated HbF and reduced HbS values than their male coun-

terparts. Several studies have reported sex-related differences in the percentage expression of hemoglobin types, particularly HbF. According to a survey by Bain (17), globin gene expression may be influenced by hormonal and genetic factors, which may explain why females typically have somewhat greater HbF levels than males. These differences persist until approximately 6 to 12 months of age, when HbF is almost entirely replaced by HbA (18). Risoluti *et al* (19) opined that the potential impact of sex-specific factors, such as androgen levels, on the regulation of hemoglobin synthesis and the variations in hemoglobin profiles between males and females must have been responsible for these gender differences in the expression of the hemoglobin types. These sex-related differences are often insignificant in magnitude, and their clinical significance may vary depending on the particular situation and underlying hemoglobin disorders under investigation (18,20). The lower HbS values in female infants are because female infants inherit one HbS gene from their affected parent (carrier mother) and one normal Hb gene from their unaffected parent (carrier father). Male infants inherit one HbS gene from their affected parent (carrier mother) and one Y chromosome from their father. The resultant effect is that female infants have lower overall HbS levels due to the presence of one normal Hb gene. Lower HbS levels in female infants reduce the risk of SCD symptoms (21).

The higher HbS levels among the male infants could be attributed to male infants inheriting one HbS gene from their affected parent (carrier mother) and one Y chromosome from their father. Female infants inherit one HbS gene from their affected parent (carrier mother) and one normal Hb gene from their unaffected parent (carrier father). As a result, male infants tend to have higher overall HbS levels due to the presence of one normal Hb gene. The implication is that the elevated HbS levels in male infants may increase the risk of SCD symptoms if both HbS genes are inherited (20, 21).

A negative correlation existed between HbA and HbS levels. This inverse relationship is supported by studies on sickle cell disease, where HbA and HbS levels are often inversely related due to the competitive synthesis pathways. Dan et al. (22) stated that in sickle cell-beta thalassemia (HbS/β-thal), the amount of HbA produced varies depending on the type of beta-thalassemia mutation. In HbS/β^0-thalassemia, HbA production is abolished, resulting in a phenotype similar to sickle cell anemia (HbSS). In HbS/β^+-thalassemia, variable amounts of HbA (ranging from <5% to 45%) dilute HbS and inhibit polymerization, leading to a milder clinical phenotype (23).

HbF and HbS showed a negative correlation, which explains Al-Shuelli *et al* (24) findings that higher HbF levels can mitigate the clinical severity of sickle cell disease by inhibiting the polymerization of HbS. This protective effect explains the negative correlation observed in our study, suggesting a potential clinical benefit of higher HbF levels in individuals with higher HbS (25).

The negative correlation between hemoglobin A (HbA) and the age of infants, as observed in this study, can be understood through the transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA). HbF and HbA Transition occurs as follows: a) At birth, infants predominantly have HbF, which accounts for approximately 80% of their hemoglobin. As they grow, HbF gradually decreases; by around 6 months of age, it is mainly replaced by HbA. HbA2, another type of hemoglobin, is also present in small amounts during this transition (24).

Concerning HbA and HbF, healthy adults primarily have significant levels of HbA and HbA2. HbF remains the primary type of hemoglobin in an unborn baby's body. Abnormal levels of HbF can indicate certain conditions, such as thalassemia or sickle cell anemia (24).

The clinical implications of these hemoglobin types in infants are that high HbF levels in infants are normal initially but decrease over time. The transition from HbF to HbA is essential for oxygen transport and overall health. Understanding this process helps manage conditions related to abnormal hemoglobin variants (13,14).

The negative correlation between HbA2 and the age of infants, as well as with HbF and HbS, can be explained by the transition from fetal hemoglobin (HbF) to adult hemoglobin (HbA) (21). HbS (sickle cell hemoglobin) is a variant associated with sickle cell anemia. HbA2 is primarily composed of alpha and delta globin chains. The negative correlation between HbA2 and HbS likely reflects the balance between these different hemoglobin types (25-27). Abnormal levels of HbF or HbA2 can indicate specific conditions, such as thalassemia or sickle cell disease.

HbA2 is a minor hemoglobin component, comprising approximately 2% to 3% of total hemoglobin in healthy adults (25). It comprises two alpha globin chains and two delta globin chains ($\alpha 2\delta 2$). Genetic factors influence HbA2 levels. The gene responsible for HbA2 production is located on chromosome 16. Males inherit one X chromosome from their mother and one Y chromosome from their father, while females inherit one X chromosome from each parent. The gene dosage effect (having two copies of the gene) may contribute to slightly higher HbA2 levels in males. Elevated HbA2 levels can be associated with certain conditions, such as beta-thalassemia trait (where HbA2 is increased) or delta-beta-thalassemia (where HbA2 is significantly elevated). However, in healthy individuals, these small gender differences in HbA2 are not clinically significant (2,14,17).

CONCLUSION

The pattern of expression of the four hemoglobin types in this study was age-dependent. Sex was not found to influence the expression of hemoglobin types in infants. There is a gradual reduction in the expression of Hemoglobin A among infants of African descent.

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References

1. Elghetany MT, Banki K. Erythrocytic disorders. In: McPherson RA, Pincus MR, eds. Henry's Clinical Diagnosis and Management by Laboratory Methods. 24th ed. Philadelphia, PA: Elsevier; 2022: chap 33.
2. Calihan J. Hematology. In: Kleinman K, Mcdaniel L, Molloy M, eds. The Harriet Lane Handbook. 22nd ed. Philadelphia, PA: Elsevier; 2021: chap 14.
3. Baird DC, Batten SH, Sparks SK. Alpha- and Beta-thalassemia: Rapid Evidence Review. Am Fam Physician. 2022;105(3):272-280.
4. Means RT. Approach to the anemias. In: Goldman L, Schafer AI, eds. Goldman-Cecil Medicine. 26th ed. Philadelphia, PA: Elsevier; 2020:chap 149.
5. Kevin E, Edmond W-T, Ambroise W. Sickle cell disease in sub-Saharan Africa: transferable strategies for prevention and care. Haematol Care Priorities Sub-Saharan Afr. 2021;8(10):E744-E755. doi:10.1016/S2352-3026(21)00191-5.
6. World Health Organization (WHO). Sickle Cell Disease: A Global Action Plan 2021-2030. World Health Organization. 2021
7. Khamphikham P, Punyamung M, Pornprasert S. Changes in HbA2 levels may signify hemoglobin defects in infants. Siriraj Med J. 2021;73(5):559-561. doi:10.33192/Smj.2021.46.
8. Brandow AM, Liem RI. Advances in the diagnosis and treatment of sickle cell disease. J Hematol Oncol. 2022;15:20. doi:10.1186/s13045-022-01237-z.
9. Ivaldi G, Barberio G, Harteveld CL, Giordano P. HbA2 Measurements in β-Thalassemia and in Other Conditions. Thalassemia Reports. 2020;4(2):1832. doi:10.4081/thal.2020.1832
10. Christensen RD, Bahr TM, Tweddell SM, Ohls RK, Henry E. Diagnosing Anemia in Neonates: An Evidence-Based Approach. NeoReviews. 2023;24(6):e343-e355. doi:10.1542/neo.24-6-e343.
11. Patra P. Sample size in clinical research, the number we need. Int J Med Sci Public Health 2012; 1(1):5-9
12. Wong P, Weerakul J, Sritippayawan S. Hemoglobin analysis in the first year of life: updated reference values and clinical implications. Mediterr J Hematol Infect Dis. 2020;12(1):e2020012. doi:10.4084/mjhid.2020.012.
13. Langer AL, Esrick EB. Beta-thalassemia: evolving treatment options beyond transfusion and iron chelation. Hematology Am Soc Hematol Educ Program. 2021;1:600-606. doi:10.1182/hematology.2021000313.
14. Khandros E, Blobel GA. Elevating fetal hemoglobin: recently discovered regulators and mechanisms. Blood. 2024;144(8):845-852. doi:10.1182/blood.2023022190.
15. Fucharoen S, Winichagoon P. The inherited diseases of haemoglobin are an emerging global health burden. Thalassemia Reports. 2021;1(12):e1. doi:10.4081/thal.2021.e1.
16. Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. Lancet Glob Health. 2024;12(1):e2-e10. doi:10.1016/S2214-109X(23)00345-2.
17. Bain BJ, Bates IM, Laffan MA. Dacie and Lewis Practical Haematology. 13th ed. Elsevier; 2024.
18. Khandros E, Blobel GA. Elevating fetal hemoglobin: recently discovered regulators and mechanisms. Blood. 2023;141(24):e123-e132. doi:10.1182/blood.2023012345.
19. Risoluti R, Colah R, Materazzi S. Frontiers in Hemoglobinopathies: New Insights and Methods. Front Mol Biosci. 2021;8:632916. doi:10.3389/fmolb.2021.632916.
20. Giardine B, Borg J, Viennas E, et al. Clinically relevant updates of the HbVar database of human hemoglobin variants and thalassemia mutations. Nucleic Acids Res. 2023;49(D1):D1192-D1196. doi:10.1093/nar/gkaa959.
21. Elghetany MT, Banki K. Erythrocytic disorders. In: Henry's Clinical Diagnosis and Management by Laboratory Methods. 2020:475-518.
22. Dan BM, Gutu BI, Severin E, Tanase VG. Innovative and Needs-led Research on Beta-Thalassemia Treatment Methods. Front. Hematol. 2023;1:1085952. doi:10.3389/frhem.2023.1085952.
23. Kunapa I, Suwanban T, Thungthong P, Chamnanchanunt S, Fucharoen S. Predicting factors of survival rates among alpha- and beta-thalassemia patients: a retrospective 10-year data analysis. Front. Hematol. 2024;3:1339026. doi:10.3389/frhem.2024.1339026
24. Al-Shuelli F, Al-Khabori MK, Al-Kindi S, et al. The optimal cut-off level for hemoglobin A2 to differentiate between sickle cell disease genotypes. Blood. 2018;132(Suppl 1):2391.
25. Sharma DC, Singhal S, Woike P, et al. Hereditary persistence of fetal hemoglobin. Asian J Transfus Sci. 2020;14(2):185-186.
26. Jeremiah Z, Alee M. Elevated mean cell volume in sickle cell anaemia: One story, too many? Sanamed. 2024;19(1):51-57. doi:10.5937/sanamed19-49277.
27. Jeremiah ZA, Nanaboemi WC, Jeremiah TA. Haemoglobin haplotypes and probable allelic frequencies of natives of Okolobiri Community, Nigeria: Population genetics study. Afr J Lab Haem Transf Sci. 2023;2(2):115-122. doi:10.59708/ajlhts.v2i2.2312.

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ORIGINAL ARTICLE

Cross-sectional study identifying the prevalence of sleep disturbances and associated risk factors in medical students

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Summary

Sleep disturbances can lead to poor academic and health outcomes. There is a gap in the current literature regarding the prevalence of sleep disturbances and their impact on various associated factors. Our study seeks to understand the prevalence of sleep disturbances, and their impact on medical students.

A cross-sectional survey was conducted at the University of Belgrade, Faculty of Medicine between April 2023 and September 2023. A sample of 70 medical students provided answers to a novel questionnaire, the BDI, and the PSQI, to evaluate lifestyle habits and demographics, depressive symptoms, and sleep quality.

Univariate linear regression identified chronic diseases, stress experienced in the past 12 months, sleep duration, difficulty falling or staying asleep, sleep pill consumption, and BDI score as strong predictors of the PSQI global score. Multivariate linear regression analysis showed that chronic diseases and depression were the most influential factors influencing participants' sleep quality.

Sleep quality in students is significantly affected by chronic diseases and depressive symptoms, highlighting the need for preventive measures.

Key words: sleep quality, depression, chronic disease, PSQI, BDI, students

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INTRODUCTION

Sleep quality and mental health are closely linked in university students and can be compromised by various external factors, potentially leading to poor academic performance and overall health outcomes. Sleep quality refers to the assessment of how well an individual sleeps and is characterized by factors such as sleep efficiency, sleep latency, sleep duration, and wake after sleep onset (1). Sleep quality is known to influence mental health, with previous studies identifying correlations between poor sleep quality and depressive symptoms (2).

Disturbances in sleep quality can create poor outcomes in individuals and are significantly influenced by psychological, social, as well as environmental factors. The Pittsburgh Sleep Quality Index (PSQI) is the standardized scale which can effectively evaluate the prevalence of disturbances in sleep quality. Additionally, a clear association has been found between sleep patterns and depressive symptoms, with 19% of study participants exhibiting depressive symptoms, as measured by the Beck Depression Inventory (BDI). These individuals also experienced lower sleep quality, increased sleep latency, and higher consumption of sleeping pills (2).

Among students, technology use – particularly internet and phone usage – is a major contributor to poor sleep quality. Internet usage among health students has been associated with increased rates of sleep quality issues and depression, by 42.3% and 8.9%, respectively (3). Phone usage has been shown through multiple previous studies to negatively impact sleep and mental health. The overarching result is that phone usage, and sometimes addiction, are highly associated with poorer sleep quality and worse mental health across the student population (4-6). Contributing factors include increased nighttime checking of one's phone (5) and hours spent on one's phone at daytime (6).

Furthermore, gender differences create a discrepancy in sleep quality and mental health in university students, as determined by a previous study in 2020, which found that females had a higher prevalence of poor sleep quality, insomnia, and daytime sleepiness by 5.5%, 6.8%, and 4.5%, respectively (7). Additionally, female students have also been shown to have a higher risk of depressive symptoms (2).

Students' academic lifestyles have been shown to contribute to the prevalence of poor sleep quality and mental health. Factors such as "all-nighters" and burnout have been linked to poor academic performance and depression (8), as well as impaired sleep quality (9).

There is limited literature on interventions for this issue; however, one study found that two cognitive-behavioral workshops – the Sleep and Wakefulness Program and the Perseverance Program – resulted in a decrease in depression both before and after workshop attendance (10). This highlights potential approaches to address this concern within the student population.

Although research on the prevalence of sleep disturbances and the impact of depression in university students is limited, previous studies have shown a clear association between the two. This underscores the need for further investigation into the factors contributing to this relationship. This association can be problematic for students' academic performance, health, and overall success, making this a pertinent problem to explore further. Keeping in mind all the above mentioned, the aim of our study was to assess the prevalence of sleep disturbances and consequent risk factors in the cohort of medical students.

MATERIAL AND METHODS

A cross-sectional, comprehensive survey was conducted at the University of Belgrade, Faculty of Medicine between April 2023 and September 2023. A sample of 70 medical students out of the total of280, in various years of study, who had their studies in English, voluntarily participated in an online survey, with their responses remaining anonymous. The survey aimed to gather information regarding students' demographics, sleep habits, presence of depressive symptoms, and overall lifestyle. The study was approved by the Institutional Review Board of the Faculty of Medicine, University of Belgrade.

A new 33-question questionnaire was developed by a member of the research team, following an initial literature review to identify the relevant and essential questions to include.

The Beck Depression Inventory (BDI) was administered to students in order to gather information on possible depressive symptoms (11). This questionnaire included 21 areas related to depression. The answers were collected, totaled, and scored by a research team member based on a scale, with 0 to 13 points = no depressive symptoms, 14 to 19 points = mild depressive symptoms, 20 to 28 points = moderate depressive symptoms, and > 28 points = severe depressive symptoms.

The Pittsburgh Sleep Quality Inventory (PSQI) was also administered to students, in order to gather information on their sleep habits, sleep disturbances, and the overall quality of sleep (12). This questionnaire contained 19 questions, which were self-rated, as well as 5 partner or roommate questions, if available. However, only the self-rated questions were scored. The 19 questions were used to form 7 component scores, with ranges of 0-3, 0 meaning no difficulties with sleep, and 3 meaning severe difficulties with sleep and high dysfunction. The component scores were subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, the use of sleeping medication, and daytime dysfunction. These component scores were added together to form the Global PSQI Score, which is indicative of the level of sleep quality, and ranges from

0-21 points, 0 being no difficulty overall, and 21 being severe difficulty in all areas mentioned above regarding sleep quality (12).

STATISTICAL ANALYSIS

The primary analysis involved descriptive summary statistics for estimating socio-demographic characteristics of participants. The level of statistical significance was set at $p<0.05$. Pearson's or Spearman's correlation coefficients (r or ρ), depending on normality of distribution were used to investigate the association between the PSQI and the BDI scores. In data analysis, univariate and multivariate linear regression models were used. All analyses were performed using Statistical Package for Social Sciences (SPSS), version 17.0.

RESULTS

Demographic and social characteristics of study participants are presented in **Table 1**. Among the study participants, there were more females than males, with an average age of 23 years. Half of the participants were born in Serbia, with Canada, USA, and Germany being countries with >1 participant. The majority of participants identified themselves as Caucasian. The majority of study participants were in their 6th year of medical school, with an average GPA of 8.3 on a 5-10 scale. Most participants reported living with their parents or in a rented apartment, and the average monthly allowance was 535 euros.

Distribution of study participants regarding their health and lifestyle habits are presented in **Table 2**. Lifestyle habits include smoking, coffee and alcohol consumption, physical activity, internet use, job positions, stress, and sleeping. The majority of study participants reported no history of chronic disease, were nonsmokers, and more than half had never consumed coffee. Alcohol consumption was varied, however most participants consumed alcohol either 1-3 times per month or less than once a month. In terms of activity, the largest proportion of study participants engaged in sports-related activities weekly, participated in recreational activities daily, and exercised 2-3 times per week, primarily through active sports or workouts. Participants spent most of their time sitting for over 8 hours daily and spent up to 5 hours of their free time on the computer. Study participants reported spending the most time on schoolwork and studying, with an average of 3-5 hours. Over 80% of participants did not have a student job outside their education, while the 20% who did worked an average of 13 hours per week. Most participants described experiencing stress occasionally over the past 12 months, and nearly 60% reported sleeping 6-8 hours per night. Study participants reported that their most common issue was difficulty falling or staying asleep, with 80% occasionally using sleeping pills.

Table 1. Demographic and social characteristics of study participants

Variable	Value
Gender	
Male	26 (37.1%)
Female	44 (62.9%)
Age (years)	
Mean±SD	23.0±2.6
Range	19-33
Country of Birth	
Serbia	35 (50%)
Canada	4 (5.7%)
USA	4 (5.7%)
Germany	3 (4.3%)
Other	24 (34.3%)
Ethnic Origin	
Caucasian	56 (80%)
Mixed ethnicity	4 (5.7%)
Asian/Pacific Islander	4 (5.7%)
Persian	2 (2.9%)
African	1 (1.4%)
Arabic	1 (1.4%)
Hispanic/Latino	1 (1.4%)
Indian	1 (1.4%)
Year of study	
First	14 (20%)
Second	8 (11.4%)
Third	7 (10%)
Fourth	16 (22.9%)
Fifth	7 (10%)
Sixth	18 (25.7%)
Grade Point Average (GPA)*	
Mean±SD	8.3±0.8
Range	6-10
Current Housing	
Living with parents	33 (47.1%)
Rented apartment	24 (34.3%)
Own apartment	10 (14.3%)
Student housing	3 (4.3%)
Amount of money received per month (EUR)	
Mean±SD	535.5±609.2
Range	0-3,000

*GPA is weighed on a scale of 5-10.

Mean and median values of PSQI scores are presented in **Table 3**. The median value of the PSQI global score was 6.0.

Depressive mood was measured by BDI, and median value of the BDI was 7.0, range 0-35. The distribution of the score categories for BDI is present in **Table 4**.

Correlation analysis showed a statistically significant relationship between the total score of PSQI and the following variables: BDI score ($\rho=0.631$, $p=0.001$), presence of chronic diseases ($\rho=0.243$, $p=0.042$), time spent sitting ($\rho=0.260$, $p=0.029$), frequency of feeling stressed in the past 12 months ($\rho=0.428$, $p=0.001$), sleep duration per night ($\rho=-0.487$, $p=0.001$), difficulties with falling asleep or stay asleep ($\rho=-0.506$, $p=0.001$).

Table 2. Distribution of study participants according to health and habits

Variable	Value
Presence of chronic diseases	
Yes	14 (20%)
No	56 (80%)
Smoking	
Nonsmokers	45 (64.3%)
Current smokers	18 (25.7%)
Past smokers	7 (10%)
Smoking duration (years)	
Mean±SD	5.2±4.4
Range	1-9
Number of cigarettes smoked per day	
Mean±SD	13.5±10.1
Range	1-40
Coffee consumption	
Daily	16 (22.9%)
Sometimes	18 (25.7%)
Never	36 (51.4%)
Alcohol consumption	
Never	13 (18.6%)
Less than once a month	21 (30%)
1-3 times/month	21 (30%)
Once a week	8 (11.4%)
2-4 times/week	7 (10%)
Frequency of sport-related activity	
Never	17 (24.3%)
Monthly	14 (20%)
Weekly	23 (32.9%)
Daily	16 (22.8%)
Frequency of recreational activity	
Rarely	16 (22.9%)
2 times per week	23 (32.9%)
Daily	31 (44.2%)
Frequency of physical activity	
Daily	21 (30%)
2 -3 times/week	26 (37.1%)
Once a week	3 (4.3%)
2-3 times/month	4 (5.7%)
Once a month	3 (4.3%)
Level of physical activity	
Active sport/workout	26 (37.1%)
Recreational exercise	20 (28.6%)
Walking	24 (34.3%)
Time spent sitting	
Up to 3 hours	3 (4.3%)
Up to 5 hours	25 (35.7%)
Up to 8 hours	29 (41.4%)
More than 8 hours	13 (18.6%)
Free time spent on computer	
1-2 hours	22 (31.4%)
Up to 5 hours	28 (40%)
More than 5 hours	12 (17.2%)
I don't use a computer in free time.	8 (11.4%)
Time spent on school work/studying	
1-3 hours	18 (25.7%)
3-5 hours	28 (40%)
5-7 hours	18 (25.7%)
More than 7 hours	6 (8.6%)
Presence of student job	
Yes	13 (18.6%)
No	57 (81.4%)
Duration of student job per week (hours)	
Mean±SD	13.1±15.1
Range	2-50
Feelings of stress over past 12 months	
Rarely	5 (7.1%)
Sometimes	40 (57.2%)
Always	25 (35.7%)
Sleep duration	
4-6 hours	21 (30%)
6-8 hours	41 (58.6%)
8-10 hours	8 (11.4%)
Difficulties falling or staying asleep	
Yes	14 (20%)
No	25 (35.7%)
Sometimes	31 (44.3%)
Consumption of sleeping pills	
Yes	8 (11.4%)
No	5 (7.2%)
Sometimes	57 (81.4%)

Table 3. Pittsburgh Sleep Quality Index (PSQI) scores according to components

Component	Mean score±SD	Median, IQR*
Subjective sleep quality	1.2±0.8	1.0, 1.0
Sleep latency	1.2±1.1	1.0, 2.0
Sleep duration	0.9±0.9	1.0, 1.0
Habitual sleep efficiency	0.3±0.7	0.0, 0.0
Sleep disturbances	1.0±0.6	1.0, 0.0
Use of sleeping medication	0.2±0.7	0.0, 0.0
Daytime dysfunction	1.1±0.8	1.0, 0.0
PSQI global score	5.8±3.1	6.0, 3.0

*IQR = interquartile range.

Table 4. The distribution of the BDI score categories

BDI Score	Number of subjects	%
0-13	57	81.5
14-19	5	7.2
20-28	6	8.5
29+	2	2.8

According to results of univariate linear regression analysis, statistically significant predictors of the PSQI global score in our study include the presence of chronic diseases, feelings of stress in the past 12 months, sleep duration, difficulties with falling or staying asleep, consumption of sleeping pills, and BDI score **(Table 5)**.

All variables found to be statistically significant in the univariate linear regression analysis were included in the multivariate model. Multivariable linear regression analysis showed that independent predictors of the PSQI global scores included the presence of chronic diseases (unstandardized beta coefficient=1.89, standard error=0.70, p=0.009) and BDI score (unstandardized beta coefficient=0.25, standard error=0.04, p=0.001), in our settings.

Table 5. Linear regression analysis

	Univariate analysis			Multivariate analysis		
Variable	Unstandardized beta coefficient	95% confidence intervals	p	Unstandardized beta coefficient	95% confidence intervals	p
Gender	0.74	-0.82, 2.29	0.347			
Age	0.22	-0.07, 0.51	0.136			
Year of study	0.11	-0.30, 0.52	0.582			
GPA	-0.88	-1.93, 0.17	0.098			
Current housing	-0.24	-1.18, 0.70	0.612			
Amount of money received per month	-0.01	-0.01, 0.0	0.074			
Presence of chronic diseases	2.34	0.53, 4.14	**0.012**	1.89	0.49, 3.29	**0.009**
Smoking	0.01	-1.12, 1.14	0.986			
Coffee consumption	0.60	-0.32, 1.51	0.200			
Alcohol consumption	0.09	-0.54, 0.73	0.771			
Frequency of sport-related activity	-0.16	-0.85, 0.53	0.647			
Frequency of recreational activity	0.44	-0.51, 1.39	0.355			
Time spent sitting	0.81	-0.11, 1.73	0.082			
Free time spent on computer	0.57	-0.20, 1.34	0.144			
Time spent on school work/studying	0.17	-0.66, 0.99	0.688			
Presence of student job	-1.61	-3.52, 0.30	0.097			
Feelings of stress	2.33	1.18, 3.48	**0.001**			
Sleep duration	-2.41	-3.49, -1.33	**0.001**			
Difficulties falling or staying asleep	-2.0	-2.87, -1.15	**0.001**			
Consumption of sleeping pills	-1.17	-2.27, -0.06	**0.039**			
BDI score	0.26	0.18, 0.34	**0.001**	0.25	0.18, 0.34	**0.001**

*Bold values denote statistical significance

DISCUSSION

Our study examined the effect of lifestyle habits and mental health, specifically depression, on the sleep quality of medical students in Belgrade, Serbia using a novel questionnaire and two indexes, the Pittsburgh Sleep Quality Index (PSQI) and the Beck depression Inventory (BDI). The mean PSQI global score identified in this population was 5.8, on the scale 0-21. Of all factors incorporated in the study, univariate linear regression analysis identified chronic diseases, feeling of stress in the past 12 months, sleep duration, difficulty with falling or staying asleep, consumption of sleep pills, and BDI score as strong predictors for the PSQI global score, which identifies the level of sleep quality. Multivariate linear regression analysis revealed that the BDI score and the presence of chronic diseases had the strongest correlation and statistical significance with the PSQI score. This indicates that chronic diseases and depression were the most influential factors affecting the study participants' sleep quality. This indicates a need to prioritize the treatment of chronic disease and depression in this population to maximize their sleep quality, which holds a heavy effect on the academic performance and success of the study participants.

Similar studies have been conducted regarding the association between mental health, technology use, and sleep quality in the student population. Dudo et al. found an association between depressive symptoms and lower sleep quality as well as high sleep latency, and the consumption of sleeping pills, similarly using the BDI and PSQI (2). This is consistent with our results, as it identifies a link in mental health and sleep quality of the medical student population. Becker et al. looked more specifically at PSQI, finding that through structural regression models, depression and anxiety were associated with PSQI sleep component disruptions, however depression specifically was uniquely associated with increased daytime dysfunction (13). While our study was consistent with the initial general association, we did not discover associations with daytime function. A study by Thacher also identified a growing trend between depressive symptoms and a single night of total sleep deprivation ("all-nighter"), further emphasizing the link between mental health and sleep quality (8). This raises the idea of how student's voluntary sleep behaviors influence their mental health and sleep quality outcomes.

We found correlations between the time spent on the computer and the BDI score in the study population, which is consistent with other studies investigating similar outcomes. For example, Kaya et al. discovered that students who used their smartphones for a mean of 7.85 hours per day had a statistically significant relationship to a higher PSQI and BDI point (6). Our results, demonstrated through correlational analysis, showed that internet usage – typically reported as 5 or more hours – was associated with the BDI score, aligning with the findings of Kaya et al. regarding the impact of technology use (6). Similarly, Shoval et al. found that frequent nighttime smartphone checking was linked to lower sleep quality. While we did not specifically investigate this, our find-

ings align with theirs, as difficulty staying asleep correlated with the PSQI global score. Students who frequently checked their phones at night struggled to maintain sleep (5). This similarly demonstrates the potential impacts of technology use on students' sleep quality.

Some studies found gender to be associated with sleep quality and depression, identifying females to have poorer sleep quality, a higher risk for depressive symptoms, and higher prevalence for insomnia and poor academic performance in females when compared to males (2, 7, 14), which was inconsistent with our results that did not identify a statistically significant correlation between gender and any of these factors.

Finally, burnout, which is a common occurrence for early phases in medical students, and sleep disorders, showed a bidirectional effect on students, including emotional exhaustion and daytime sleepiness, measured through PSQI and BDI scores, according to a study done by Pagnin et al (9). This highlights the impact of student lifestyle on sleep quality and mental health, and while we did not investigate burnout, it is important to note this finding when viewing our results and conducting future studies, taking into consideration student lifestyle habits based on education curriculum.

This study was conducted at a single medical faculty in Belgrade, Serbia. While the curriculum is standard across Serbian faculties and can be applied to other institutions within the country, it differs from those in other countries, which may limit the generalizability of the findings to student populations with different educational systems. Future studies should include comparisons across countries to provide more widely applicable insights.

Also, this study included a relatively low sample size of 70 students, making results limited in their external validity and generalizability. To increase the statistical power of the results, a larger sample size is needed for future studies.

This study was conducted at a specific point in students' lives, and may not capture events or emotions that could influence the results. Future research should consider a longitudinal approach to account for these factors over time.

Finally, the data collected in this study was self-reported, making the data less reliable compared to studies in which data is not self-reported. While the PSQI and BDI are established indexes, the demographic novel questionnaire is not, therefore this causes a potential limitation in data reliability. For future studies, an external measure of self-reported reliability is necessary to ensure accurate findings.

CONCLUSION

Sleep quality in the medical student population is heavily influenced by chronic diseases and depressive symptoms, as shown by multivariate linear regression analysis. Therefore, it is necessary to address symptoms of chronic diseases and depression properly and immediately, as well as apply preventative measures, for students to have improved sleep quality, and as a result, improved learning and academic performance.

Author Contributions

TP conceptualized the investigation and developed the methodology; SDJ collected data and created a database; AJ and VN performed statistical analysis; SDJ wrote the paper; all authors revised it for important intellectual content and approved the final submission.

References

1. Nelson KL, Davis JE, Corbett CF. Sleep quality: An evolutionary concept analysis. Nurs Forum. 2022;57(1):144-51, doi: 10.1111/nuf.12659
2. Dudo K, Ehring E, Fuchs S, Herget S, Watzke S, Unverzagt S, Frese T. The association of sleep patterns and depressive symptoms in medical students: a cross-sectional study. BMC Res Notes. 2022;15(1):109, doi: 10.1186/s13104-022-05975-8
3. Paudel L, Sharma P, Kadel AR, Lakhey K, Singh S, Khanal P, et al. Association Between Internet Addiction, Depression and Sleep Quality Among Undergraduate Students of Medical and Allied Sciences. J Nepal Health Res Counc. 2021;19(3):543-9, doi: 10.33314/jnhrc.v19i3.3599
4. Okasha T, Saad A, Ibrahim I, Elhabiby M, Khalil S, Morsy M. Prevalence of smartphone addiction and its correlates in a sample of Egyptian university students. Int J Soc Psychiatry. 2022;68(8):1580-8, doi: 10.1177/00207640211042917
5. Shoval D, Tal N, Tzischinsky O. Relationship of smartphone use at night with sleep quality and psychological well-being among healthy students: A pilot study. Sleep Health. 2020;6(4):495-7, doi: 10.1016/j.sleh.2020.01.011
6. Kaya F, Bostanci Daştan N, Durar E. Smart phone usage, sleep quality and depression in university students. Int J Soc Psychiatry. 2021;67(5):407-14, doi: 10.1177/0020764020960207
7. Marta OFD, Kuo SY, Bloomfield J, Lee HC, Ruhyanudin F, Poynor MY, et al. Gender differences in the relationships between sleep disturbances and academic performance among nursing students: A cross-sectional study. Nurse Educ Today. 2020;85:104270, doi: 10.1016/j.nedt.2019.104270
8. Thacher PV. University students and "the all nighter": correlates and patterns of students' engagement in a single night of total sleep deprivation. Behav Sleep Med. 2008;6(1):16-31, doi: 10.1080/15402000701796114.
9. Pagnin D, de Queiroz V, Carvalho YT, Dutra AS, Amaral MB, Queiroz TT. The relation between burnout and sleep disorders in medical students. Acad Psychiatry. 2014;38(4):438-44, doi: 10.1007/s40596-014-0093-z
10. Ehrampoush MH, Tabei SZ, Mahmoodabad SSM, Fallahzadeh H, Nami M, Khayer E, et al. A study of comparing two cognitive-behavioral workshop for college students: Sleep, wakefulness program and perseverance program. J Family Med Prim Care. 2019;8(3):1222-6, doi: 10.4103/jfmpc.jfmpc_130_19
11. Beck AT, Beck RW. Screening depressed patients in family practice. A rapid technic. Postgrad Med. 1972;52(6):81-5, doi: 10.1080/00325481.1972.11713319

12. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213, doi: 10.1016/0165-1781(89)90047-4

13. Becker SP, Jarrett MA, Luebbe AM, Garner AA, Burns GL, Kofler MJ. Sleep in a large, multi-university sample of college students: sleep problem prevalence, sex differences, and mental health correlates. Sleep Health. 2018;4(2):174-81, doi: 10.1016/j.sleh.2018.01.001

14. Surani AA, Zahid S, Surani A, Ali S, Mubeen M, Khan RH. Sleep quality among medical students of Karachi, Pakistan. J Pak Med Assoc. 2015;65(4):380-2.

ISPITIVANJE PREVALENCIJE POREMEĆAJA SPAVANJA I UDRUŽENIH FAKTORA RIZIKA KOD STUDENATA MEDICINE

Sofija Đordjević[1], Aleksa Jovanović[1], Vladimir Nikolić[1], Tatjana Pekmezović[1]

Sažetak

Poremećaji spavanja mogu dovesti do slabijih akademskih i zdravstvenih ishoda. Prevalencija poremećaja spavanja i njihov uticaj na različite aspekte života još uvek nisu dovoljno poznati. Cilj ove studije je procena prevalencije poremećaja spavanja i efekata na zdravlje koje oni imaju, kod studenata medicine.

Metod: Istraživanje po tipu studije preseka sprovedeno je na Medicinskom fakultetu Univerziteta u Beogradu, u periodu od aprila do septembra 2023. Godine. Uzorak je činilo 70 studenata medicine koji su popunili odgovarajući upitnik, The Pittsburgh Sleep Quality Inventory (PSQI) i Beckovu skalu depresivnosti (BDI), kako bi se procenile životne navike i demografske karakteristike, kvalitet spavanja i simptomi depresivnosti.

Rezultati: Univarijantnom linearnom regresionom analizom pokazano je da su hronične bolesti, prisustvo stresa u poslednjih 12 meseci, trajanje sna, teškoće pri uspavljivanju, korišćenje lekova za spavanje i BDI skor bili značajni prediktori globalnog skora PSQI. Multivarijantna linearna regresiona analiza pokazala je da su hronične bolesti i depresija najznačajniji faktori koji utiču na kvalitet spavanja kod studenata.

Zaključci: Na kvalitet spavanja kod studenata u velikoj meri utiču hronične bolesti i simptomi depresivnosti, zbog čega je neophodno prevenirati i lečiti ove zdravstvene probleme.

Ključne reči: kvalitet spavanja, depresija, hronične bolesti, PSQI, BDI, studenti

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ORIGINAL ARTICLE

Safety of intravenous thrombolysis in stroke mimics - a 15-year experience

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Summary

Introduction: The necessity of timely administration of intravenous thrombolysis (IVT) in the treatment of acute ischemic stroke may result in its administration in conditions that mimic stroke with their clinical presentation ("stroke mimics").

Aim: To assess the safety of IVT administration in stroke mimics.

Material and methods: A retrospective study of 764 patients treated with IVT was conducted at the Department of Emergency Neurology of the University Clinical Centre of Serbia in the period between 2006 and 2021.

Results: Out of 764 stroke patients treated with IVT, the diagnosis of stroke mimics was established in 22 patients (2.9%). The average age of patients with stroke mimics was 49.1 ± 16.3 (min 26, max 86), and 55% of patients were female. The most frequent vascular risk factor in this group of patients was arterial hypertension (41%), followed by smoking (14%) and hypercholesterolemia (14%). In comparison with the control group of thrombolysed patients with stroke (matched according to sex and age), a trend towards a milder initial clinical deficit measured by NIHSS score was determined (5 vs. 9; p=0.058) in the stroke mimics group. Furthermore, a higher probability of favorable functional outcome (mRS 0-2) was registered (91 % vs. 55%, p=0.007). There were no statistically significant differences between the two groups regarding the death outcome (p=1.000). Symptomatic intracerebral hemorrhage was not registered in any patient in the stroke mimics group.

Conclusion: The use of intravenous thrombolysis in acute conditions presenting with the clinical picture of acute ischemic stroke is safe for the patients.

Key-words: acute ischemic stroke, stroke mimics, intravenous thrombolytic therapy (IVT)

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INTRODUCTION

Acute ischemic stroke (AIS) is a sudden, focal, non-convulsive neurological deficit resulting from vascular damage and it represents a significant medical challenge with a noticeable increase in incidence within the general population, especially in low-income countries (1). The decision regarding the type of treatment during its hyperacute phase is determined by clinical presentation, neuroimaging diagnostic findings and the time that passed from symptom onset to patient's arrival at the hospital (2). The only therapeutic approach proven to be efficient in AIS treatment consists of timely recanalization of occluded blood vessels by intravenous thrombolysis and/or mechanical thrombectomy. Intravenous thrombolytic therapy (IVT) refers to the intravenous administration of recombinant tissue plasminogen activator (rt-PA) within a time limit of 4.5 hours from symptom onset (3,4). Furthermore, additional absolute contraindications such as already established ischemia on computed tomography (CT) scan, previous intracranial hemorrhage, current severe uncontrolled hypertension, recent intracranial/spinal surgery or head trauma restrict the use of IVT (4, 5). Nevertheless, the most important factor limiting IVT administration is time, since patients have to reach the hospital within a narrow time frame. Moreover, the benefit of IVT declines as time passes from symptom onset (4,5).

Rapid clinical assessment and brain imaging are essential when a patient presents with symptoms of an acute stroke. In many countries and hospitals only non-contrast CT scan is available in the emergency setting and is typically conducted as the initial neuroimaging procedure in patients suspected of having AIS (6). More sophisticated neuroimaging procedures such as CT perfusion scan and diffusion-weighted MRI (DWI-MRI) sequences, which are more useful imaging modalities for providing valuable support in addition to clinical suspicion in differentiating stroke from stroke mimics, are not widely available in emergency settings. Various conditions and diseases, which lack the pathoanatomical substrate of AIS can mimic its clinical presentation, potentially complicating treatment decisions (7). Considering the urgency of decision making, neurologists who must make fast yet patient-safe decisions, can find themselves in this challenging situation.

The aim of this study was to evaluate the safety of IVT administration in patients presenting with acute conditions with the same or similar clinical presentation to AIS, i.e. those diagnosed with stroke mimics.

MATERIAL AND METHODS

A retrospective analysis was conducted on data from a total of 764 patients treated with IVT at the Emergency Neurology Department of the Neurology Clinic, University Clinical Center of Serbia, between 2006 and 2021. Retrospective and completely anonymous data were used, therefore signed informed consent was not requested.

Patients' demographic characteristics were analyzed, as well as vascular risk factors: hypertension, diabetes mellitus (DM), atrial fibrillation (AF), hypercholesterolemia, smoking, previous coronary disease (myocardial infarction, peripheral arterial disease, etc.), and previous stroke. The use of prestroke medication was also evaluated, including previous antiplatelet/anticoagulant, antihypertensive and statin therapy. Data were obtained through patient history, heteroanamnesis, and review of medical records.

In all patients, evaluation of clinical deficit's severity was assessed by using the National Institutes of Health Stroke Scale score (NIHSS), which was performed upon admission (8). Afterwards, all patients had their blood pressure measured, an electrocardiogram performed, underwent a non-contrast CT scan, and had a comprehensive laboratory tests analysis. Based on clinical and neurological findings, patients with suspected AIS that met all the necessary criteria, according to the recommendations from current European and North American guidelines for the management of acute stroke, were then treated with recanalization therapy (4,9,10). The initial clinical diagnosis of AIS was then confirmed by a follow-up CT/MR neuroimaging performed 12-72 hours upon admission, depending on the administered therapy, and in case of exacerbation even earlier. Symptomatic intracerebral hemorrhage (sICH) was defined according to the ECASS 2 criteria (4). The diagnosis of stroke mimics was made according to the criteria established by Hand et al. (11). Stroke mimics were identified within the patients whose clinical presentations were not confirmed with a vascular etiology, but rather, additional examinations revealed the presence of an alternative diagnosis (12). Patients' follow-up assessment was conducted 3 months after hospitalization, by using the modified Rankin Scale (mRS). Favorable outcome was defined as a mRS score 0-2, while a fatal outcome was defined as a mRS 6 (4).

Furthermore, patients identified as stroke mimics were matched with a control group of patients diagnosed with AIS (gender and age matching) for additional analysis.

The data were analyzed by using descriptive statistical methods which included measures of central tendency (mean, median, percentiles), measures of variability (standard deviation), and structural indicators expressed as percentages. To compare the groups, Pearson's chi-square test or Fisher's exact test was used for categorical data, while Student's t-test or Mann-Whitney U test was used for numerical data. The data were analyzed using the SPSS 22.0 statistical software (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp; 2017). Statistical hypothesis testing was performed at a significance level (alpha level) of 0.05.

RESULTS

Out of 764 patients treated with IVT, the diagnosis of stroke mimics was made in 22 patients (2.9%). The average age of patients diagnosed with stroke mimics was 49.1 ± 16.3 (min 26, max 86) years, 55% being female. The most common vascular risk factor in this group was hypertension (41% of cases), followed by smoking (14%) and hypercholesterolemia (14%). The most frequently used chronic therapy in this subgroup of patients was antihypertensive therapy (36.4%). Details are presented in **Table 1**.

Table 1. Vascular risk factors and therapy for primary prevention in patients diagnosed with stroke mimics

Feature	Stroke mimics (n=22)
Hypertension, n (%)	9 (40.9%)
Diabetes mellitus, n (%)	0 (0%)
Atrial fibrillation, n (%)	1 (4.5%)
Hypercholesterolemia, n (%)	3 (13.6%)
Prior coronary disease, n (%)	0 (0%)
Prior stroke, n (%)	1 (4.5%)
Current smoking, n (%)	3 (13.6%)
Prior antihypertensive therapy, n (%)	8 (36.4%)
Prior antithrombotic therapy, n (%)	2 (9.1%)
Prior statin therapy, n (%)	2 (9.1%)

Abbreviations: n= number;

There was a trend towards less severe clinical presentation measured by NIHSS score in the stroke mimics group compared to the group of patients with AIS, but without reaching a statistical significance (5 [IQR 5-10] vs. 9 [IQR 6-19], p=0.058). There was no difference between two groups regarding systolic blood pressure (p=0.07), diastolic blood pressure (p=0.104), or glucose levels (6.0 vs. 6.7; p=0.131). On the other hand, patients with stroke mimics were more likely to have excellent functional outcome (91% vs. 41%; p<0.001), as well as favorable outcome (91% vs. 55%; p=0.007) after three months. Furthermore, it was shown that none of the patients diagnosed with stroke mimics experienced symptomatic intracerebral hemorrhage whereas its prevalence in the AIS group was 0.1% (p<0.001). Additionally, there were no fatal outcomes among the thrombolysed patients in the stroke mimics group, compared to one fatality in the AIS group, which did not constitute a statistically significant difference (p=1.000). Details are presented in **Table 2** and **Figure 1**.

Table 2. Baseline findings and outcome of IVT use in patients with stroke mimics vs. acute ischemic stroke patients

Feature	Stroke mimics (n=22)	AIS (n=22)	p -value
Systolic blood pressure, mmHg, mean ± sd	133.2 ± 19.7	144.2 ± 19.5	0.070
Diastolic blood pressure, mmHg mean ± sd	82.0 ± 12.7	87.7 ± 10.2	0.104
NIHSS, median [IQR]	5.0 (5-10.0)	9.0 (6-19)	0.058
Baseline glucose, mmol/l, mean ± sd	6.0 ± 1.4	6.7 ± 1.8	0.131
sICH, n (%)	0 (0%)	1 (4.5%)	1.000
Death outcome, n (%)	0 (0%)	1 (4.5%)	1.000
Excellent functional outcome (mRS 0–1), n (%)	20 (90.9%)	9 (40.9%)	<0.001
Good functional outcome (mRS 0–2), n (%)	20 (90.9%)	12 (54.5%)	0.007

Abbreviations: AIS = Acute ischemic stroke; SD= Standard deviation; NIHSS = National Institutes of Health Stroke Scale; IQR= Interquartile range; sICH = Symptomatic intracranial hemorrhage; n= number; mRS = Modified Rankin Scale

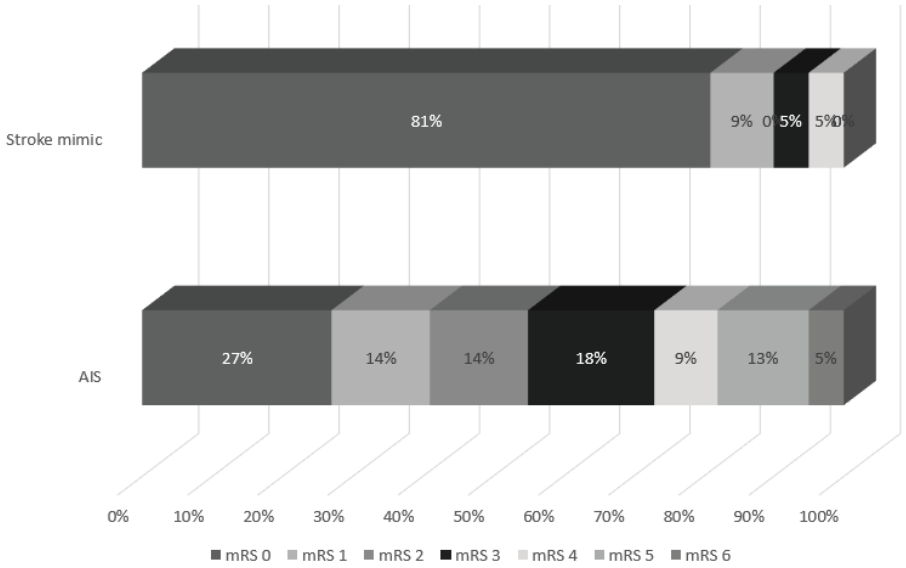

Figure 1. Three-month functional outcome of IVT treated patients with stroke mimics vs. patients with acute ischemic stroke (presented by mRS score)

Abbreviations: AIS = acute ischemic stroke; mRS= modified Rankin Scale

DISCUSSION

Although significant progress has been made in the treatment of AIS, it remains one of the leading causes of disability and death worldwide (13). Furthermore, due to the rising average age of the population and the prevalence of various comorbidities among elderly, it is anticipated that the general population will face an increased burden from AIS in the years ahead, with the assumption that one in four individuals will experience AIS (14). It has been demonstrated that the benefit of IVT rapidly declines over time from symptom onset (15). It has been estimated that every minute without appropriate treatment results in the loss of approximately 1.8 million neurons, emphasizing the importance of minimizing the time period from the onset of symptoms to IVT administration (16).

The proportion of stroke mimics in the group of patients treated with intravenous thrombolytic therapy in our study was 2.9%. Previously published studies reported the frequency of IVT treated stroke mimics ranging from 1.4% to 15.5% (17-26). One potential explanation for such a low proportion in our study is the fact that it was conducted in one of the largest health care centers in the country, where doctors have extensive experience in IVT administration decision-making process. Their clinical experience in treating patients with AIS is valuable for the outcome of the treatment itself. It has been reported that the proportion of patients with stroke mimics is higher in hospitals where the decision to administer IVT is made based on telephone consultations between emergency medicine doctors, who are the first one to examine the patient, and on-call neurologists (24). Although it is known that MRI is more sensitive in detecting potential stroke mimics (24), it is still not routinely performed in most health care centers, and the diagnosis of AIS remains, above all, a clinical diagnosis combined with a non-contrast CT scan (12), as was the case in this study.

Our study showed that none of the patients with stroke mimics developed symptomatic intracerebral hemorrhage while its incidence in the group of AIS patients was 4.5% (p=1.000). Regarding mortality outcomes, there were no deaths in the stroke mimics group, as opposed to one death in the AIS group, which was not a statistically significant difference (p=1.000). These results are consistent with those of a large multicenter international study, which also demonstrated the safety of IVT use in stroke mimics, with a low incidence of complications (12). That study reported the incidence of sICH in the stroke mimics group of 1% (95% CI 1.5-2.2) vs. 7.9% (95% CI 7.2-8.7) in AIS group, while the mortality rate in the stroke mimics group was 2.1% (95% CI 0.3-7.3) vs. 14.4% (95% CI 13.4-15.3) in the group of patients with acute ischemic stroke (12). The cited study also showed a statistically significant difference between the two groups in terms of excellent functional outcome (mRS 0-1) and good functional outcome (mRS 0-2), in both cases, p<0.0001 (12), which is consistent with our results (mRS 0-1 p=0.001, mRS 0-2 p=0.007). Although, ideally, IVT should not be administered to patients without a pathological substrate for acute ischemic stroke, the aforementioned results highlight that the safety of patients with stroke mimics is not compromised by the use of IVT. Moreover, numerous studies have shown that the benefit of early IVT administration is correlated with better outcomes in the treatment of patients with AIS (27, 28, 29). Therefore, in uncertain situations, it is entirely reasonable to administer IVT until an additional/more sophisticated diagnostic method can exclude the diagnosis of AIS (12).

The main limitation of this study lies in the fact that it was conducted at a single health care center, specifically a tertiary healthcare facility with extensive experience, resulting in a relatively small number of patients diagnosed with stroke mimics through retrospective evaluation. Taking this into account, it is assumed that the results could potentially be less favorable in smaller centers. Certainly, one of the plans for future analysis would be to include a larger number of national centers in order to obtain a more comprehensive picture of the frequency of stroke mimics among IVT treated patients.

CONCLUSION

Although the use of intravenous thrombolysis is often still accompanied by some apprehension, our study has demonstrated that IVT administration in acute conditions presenting with a clinical picture of acute ischemic stroke is safe for the patient. While it remains crucial to adhere to clinical guidelines when administering IVT, as well as to continually improve diagnostic approaches for patients with AIS, the results are encouraging. These findings may potentially serve as a rationale for neurologists to confidently initiate IVT in a timely manner, as the risk of complications from administered therapy is minimal, even in cases of stroke mimics.

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Conflicts of interest:

DRJ received speaker honoraria from Medtronic and Boehringer Ingelheim.
VP received speaker honoraria from Medtronic and Boehringer Ingelheim. Other authors declare no conflict of interest related to the presented study.

Ethical approval

In this paper, only retrospective data were used and obtained from medical records, thus Ethical approval was not obtained. Corresponding author and co-authors undertake that this research's processed data are presented in a way that does not allow the individual subject's identification. All data are related exclusively to the topic of research, without possibility of connecting the data with the identity of persons.

REFERENCES

1. Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. Lancet Neurol. 2009; 8(4):355-69. doi: 10.1016/S1474-4422(09)70025-0. PMID: 19233729.
2. Mendelson SJ, Prabhakaran S. Diagnosis and Management of Transient Ischemic Attack and Acute Ischemic Stroke: A Review. JAMA. 2021; 325(11):1088-1098. doi:10.1001/jama.2020.26867. PMID: 33724327.
3. Fugate JE, Rabinstein AA. Absolute and Relative Contraindications to IV rt-PA for Acute Ischemic Stroke. Neurohospitalist. 2015; 5(3):110-21. doi: 10.1177/1941874415578532. PMID: 26288669.
4. Berge E, Whiteley W, Audebert H, De Marchis GM, Fonseca AC, Padiglioni C, et al. European Stroke Organisation (ESO) guidelines on intravenous thrombolysis for acute ischaemic stroke. Eur Stroke J. 2021; 6(1):I-LXII. doi: 10.1177/2396987321989865. PMID: 33817340.
5. Stolper E, Van de Wiel M, Van Royen P, Van Bokhoven M, Van der Weijden T, Dinant GJ. Gut feelings as a third track in general practitioners' diagnostic reasoning. J Gen Intern Med. 2011; 26(2):197-203. doi: 10.1007/s11606-010-1524-5. PMID: 20967509.
6. Vymazal J, Rulseh AM, Keller J, Janouskova L. Comparison of CT and MR imaging in ischemic stroke. Insights Imaging. 2012; 3(6):619-27. doi: 10.1007/s13244-012-0185-9. PMID: 23055115.
7. Yew KS, Cheng EM. Diagnosis of acute stroke. Am Fam Physician. 2015; 91(8):528-36. PMID: 25884860.
8. Kwah LK, Diong J. National Institutes of Health Stroke Scale (NIHSS). J Physiother. 2014; 60(1):61. doi: 10.1016/j.jphys.2013.12.012. PMID: 24856948.
9. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/ American Stroke Association. Stroke. 2019; 50(12):e344-e418. doi: 10.1161/ STR. 0000000000000211. PMID: 31662037.
10. Turc G, Bhogal P, Fischer U, Khatri P, Lobotesis K, Mazighi M, et al. European Stroke Organisation (ESO) - European Society for Minimally Invasive Neurological Therapy (ESMINT) Guidelines on Mechanical Thrombectomy in Acute Ischemic Stroke. J Neurointerv Surg. 2023; 15(8):e8. doi: 10.1136/neurintsurg-2018-014569. PMID: 30808653.
11. Hand PJ, Kwan J, Lindley RI, Dennis MS, Wardlaw JM. Distinguishing between stroke and mimic at the bedside: the brain attack study. Stroke. 2006; 37(3):769-75. doi: 10.1161/01.STR.0000204041.13466.4c. PMID: 16484610.
12. Zinkstok SM, Engelter ST, Gensicke H, Lyrer PA, Ringleb PA, Arto V, et al. Safety of thrombolysis in stroke mimics: results from a multicenter cohort study. Stroke. 2013; 44(4):1080-4. doi: 10.1161/ STROKEAHA.111.000126. PMID: 23444310.
13. Mosconi MG, Paciaroni M. Treatments in Ischemic Stroke: Current and Future. Eur Neurol. 2022; 85(5):349-366. doi: 10.1159/000525822. PMID: 35917794.
14. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol. 2021; 335:113518. doi: 10.1016/j.expneurol.2020.113518. PMID: 33144066.
15. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, et al. ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med. 2008; 359(13):1317-29. doi: 10.1056/NEJMoa0804656. PMID: 18815396.
16. Lees KR, Bluhmki E, von Kummer R, Brott TG, Toni D, Grotta JC, et al. Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. Lancet. 2010; 375(9727):1695-703. doi: 10.1016/ S0140-6736(10)60491-6. PMID: 20472172.
17. Artto V, Putaala J, Strbian D, Meretoja A, Piironen K, Liebkind R, et al. Stroke mimics and intravenous thrombolysis. Ann Emerg Med. 2012; 59(1):27-32. doi: 10.1016/j.annemergmed.2011.09.011. PMID: 22000770.
18. Chen Y, Bogosavljevic V, Leys D, Jovanovic D, Beslac-Bumbasirevic L, Lucas C. Intravenous thrombolytic therapy in patients with stroke mimics: baseline characteristics and safety profile. Eur J Neurol. 2011; 18(10):1246-50. doi: 10.1111/j.1468- 1331.2011.03367.x. PMID: 21366806.
19. Chernyshev OY, Martin-Schild S, Albright KC, Barreto A, Misra V, Acosta I, et al. Safety of tPA in stroke mimics and neuroimaging-negative cerebral ischemia. Neurology. 2010; 74(17):1340-5. doi: 10.1212/ WNL.0b013e3181dad5a6. PMID: 20335564.
20. Förster A, Griebe M, Wolf ME, Szabo K, Hennerici MG, Kern R. How to identify stroke mimics in patients eligible for intravenous thrombolysis? J Neurol. 2012; 259(7):1347-53. doi: 10.1007/s00415-011-6354-9. PMID: 22231865.
21. Giraldo EA, Khalid A, Zand R. Safety of Intravenous Thrombolysis within 4.5 h of symptom onset in patients with negative post-treatment stroke imaging for cerebral infarction. Neurocrit Care. 2011; 15(1):76-9. doi: 10.1007/s12028-011-9523-x. PMID: 21394544.
22. Scott PA, Silbergleit R. Misdiagnosis of stroke in tissue plasminogen activator-treated patients: characteristics and outcomes. Ann Emerg Med. 2003; 42(5):611-8. doi: 10.1016/s0196-0644(03)00443-8. PMID: 14581912.
23. Tsivgoulis G, Alexandrov AV, Chang J, Sharma VK, Hoover SL, Lao AY, et al. Safety and outcomes of intravenous thrombolysis in stroke mimics: a 6-year, single-care center study and a pooled analysis of reported series. Stroke. 2011; 42(6):1771-4. doi: 10.1161/STROKEAHA.110.609339. PMID: 21493900.
24. Uchino K, Massaro L, Hammer MD. Transient ischemic attack after tissue plasminogen activator: aborted stroke or unnecessary stroke therapy? Cerebrovasc Dis. 2010; 29(1):57-61. doi: 10.1159/000255975. PMID: 19893313.
25. Winkler DT, Fluri F, Fuhr P, Wetzel SG, Lyrer PA, Ruegg S, et al. Thrombolysis in stroke mimics: frequency, clinical characteristics, and outcome. Stroke. 2009; 40(4):1522-5. doi: 10.1161/STROKEAHA.108.530352. PMID: 19164790.
26. Chang J, Teleb M, Yang JP, Alderazi YJ, Chapple K, Frey JL, et al. A model to prevent fibrinolysis in patients with stroke mimics. J Stroke Cerebrovasc Dis. 2012 Nov;21(8):839-43. doi: 10.1016/j.jstrokecerebrovasdis.2011.04.018. PMID: 21640609.
27. IST-3 collaborative group; Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, Murray G, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. Lancet. 2012; 379(9834):2352-63. doi: 10.1016/S0140-6736(12)60768-5. PMID: 22632908.
28. Ragoschke-Schumm A, Walter S, Haass A, Balucani C, Lesmeister M, Nasreldein A, et al. Translation of the 'time is brain' concept into clinical practice: focus on prehospital stroke management. Int J Stroke. 2014; 9(3):333-40. doi: 10.1111/ijs.12252. PMID: 24593156.

29. Chen CH, Tang SC, Tsai LK, Hsieh MJ, Yeh SJ, Huang KY, et al. Stroke code improves intravenous thrombolysis administration in acute ischemic stroke. PLoS One. 2014; 9(8):e104862. doi: 10.1371/journal.pone.0104862. PMID: 25111200.

BEZBEDNOST PRIMENE INTRAVENSKE TROMBOLITIČKE TERAPIJE KOD STANJA KOJA IMITIRAJU MOŽDANI UDAR ("STROKE MIMICS") - PETNAESTOGODIŠNJE ISKUSTVO

Uroš Miladinović [1], Rea Mikulan [1], Dejana R. Jovanović [1,2], Višnja Pađen [1,2]

Sažetak

Uvod: Neophodnost donošenja brze odluke o primeni intravenske trombolize (IVT) u lečenju akutnog ishemijskog moždanog udara (AIMU) može imati za posledicu njeno ordiniranje i kod stanja koja svojom kliničkom slikom mogu da imitiraju AIMU.

Cilj: Ispitivanje bezbednosti primene IVT kod drugih akutnih stanja koji se prezentuju istom ili sličnom kliničkom slikom kao AIMU, tj. "stroke mimics".

Materijal i metode: Sprovedena je retrospektivna analiza podataka ukupno 764 pacijenta lečenih trombolitičkom terapijom na Odeljenju urgentne neurologije, Klinike za neurologiju Univerzitetskog kliničkog Centra Srbije u periodu od 2006. do 2021. godine.

Rezultati: Od ukupno 764 pacijenta sa AIMU lečenih primenom IVT, dijagnoza "stroke mimics" je postavljena kod 22 pacijenta (2.9%). Prosečna starost grupe pacijenata sa dijagnozom "stroke mimics" je iznosila 49,1 ± 16,3 (min 26, max 86), od čega je 55% pacijenata bilo ženskog pola. Najučestaliji vaskularni faktor rizika prisutan u ovoj grupi pacijenata bila je arterijska hipertenzija (41%), potom pušenje (14%) i hiperholesterolemija (14%). Poređenjem ovih pacijenata sa kontrolnom grupom tromboliziranih pacijenata sa AIMU (uparenih prema polu i starosti) utvrđeno je postojanje trenda ka blažem inicijalnom kliničkom deficitu merenom NIHSS skorom (5 vs. 9; p=0.058), uz veću verovatnoću nastanka povoljnog funkcionalnog ishoda (mRS 0-2) (91% *vs*. 55%; p=0.007) u "stroke mimics" grupi. Ni kod jednog pacijenta sa "stroke mimcs" nije utvrđena pojava simptomatske intracerebralne hemoragije. Između dve grupe nije utvrđeno postojanje statistički značajne razlike kada je u pitanju pojava smrtnog ishoda (p=1.000).

Zaključak: Upotreba IVT kod akutnih stanja koja se prezentuju kliničkom slikom AIMU je bezbedna po pacijenta.

Ključne reči: akutni ishemijski moždani udar (AIMU), "stroke mimics", intravenska trombolitička terapija (IVT)

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ORIGINAL ARTICLE

Sex-specific risk factor awareness, covert misogyny and long-term cardiovascular risk management: pilot study

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Summary

INTRODUCTION: Health literacy is a cornerstone of prevention, while sex specific prevention of cardiovascular disease, a leading cause of death of women worldwide, remains less addressed and is still more a matter of local cultural habits than guideline-directed management.

METHODS: A validated multiple-choice questionnaire (MCQ) designed to both educate patients and help the healthcare team learn about their traditional and sex specific modern risk factors (RF) management, including opting for comprehensive personalized long term follow up (FU) was offered to all in-patients.

RESULTS: Out of 130 patients hospitalized at our department (Jan 1, 2024 – Feb 24, 2024) who were offered to fill in the MCQ, 37.7% accepted to do so, while 11.5% were excluded on ethical grounds. Women (49%) were older than men, had a more significant burden of traditional RF and possessed higher levels of knowledge and interest in RFs of both sexes, while men – especially those who declined to participate without a clear reason – even stated having no interest in their female relatives' risk profiles. Men, unlike women, showed no particular interest in a personalized and tech-savvy options of FU, although they equally use hand-held devices.

CONCLUSION: These results confirm the findings obtained from a similar study conducted on an all-female sample in the same setting a year ago. However, results in men were surprising for the hostility exhibited towards junior and mid-career women which was absent when approached by a senior male member of the healthcare team. This confirms that misogyny needs to be actively suppressed.

Key words: health literacy, heart disease, cardiovascular risk, sex specific risk factors, misogyny

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INTRODUCTION

Cardiovascular disease remains the leading cause of morbidity and mortality in women and men, worldwide. Most precise statistics are offered annually by the American Heart Association (1) that recently started addressing not only United States' statistics, but also global statistics linked to both cardiovascular and cerebrovascular diseases. This report also highlights the status of the AHA's "Essential Eight" risk factors (2), which include traditional factors – glycemia, cholesterol, and blood pressure levels-as well as lifestyle factors such as smoking, physical activity, diet, weight, and sleep habits, all of which collectively shape cardiovascular health. Risk factors were first identified based on findings from two major epidemiological registries. The **Seven Countries Study (SCS)** included only men from seven countries across three continents – the United States, Japan, Finland, the Netherlands, Italy, Greece, and former Yugoslavia (with Serbia's three cohorts continuing follow-up for up to 55 years). At the time, in the post-WWII era, women were believed to be protected from heart disease until well into menopause. The **Framingham Heart Study (FHS)**, in contrast, included both men and women, though primarily of Caucasian descent. Notably, while maternal morbidity and mortality data were collected for SCS participants, this later proved to be both insightful and valuable. A 40-year follow-up revealed that men whose mothers had hypertension and/or heart disease experienced worse cardiovascular outcomes in terms of both morbidity and mortality (3). However, it was the **Nurses' Health Study (NHS)** that first established a link between night shift work and an increased risk of developing diabetes (4) and obesity (5) – both of which, over time, contribute to a higher incidence and progression of cardiovascular and cerebrovascular disease. This association was further reinforced by findings from a 24-year follow-up, which demonstrated poorer overall health outcomes with aging (6). Sex specific risk factors, however, reached the guidelines only in the 21st century with the American guidelines being the first to include them in 2019 (7), while European ones followed 2 years later (8). Locally, despite coordinated efforts from all stakeholders (9) to provide comprehensive cardiovascular care for women in Serbia based on established global models (10, 11), a major barrier persists: a form of covert misogyny that has yet to be quantified in national samples. Clinically, it closely resembles the systemic racism faced by women of color in accessing reproductive care and in experiencing higher peri- and postpartum morbidity and mortality – disparities that persist even among those with no barriers to healthcare access or insurance (12, 13). This stems from the misperception that sex-specific care beyond reproductive and oncological health is an extension of militant feminism rather than a medical necessity. The global need for a comprehensive approach is the central theme of *The Lancet* Women and Cardiovascular Disease Commission (14), which, in its latest revision, highlighted the impact of discrimination that endangers over 50% of the world's population. This issue has also been recently emphasized by leading experts in the field (15) and is the focus of the latest call to action (16), which seeks to improve risk stratification for young women at higher risk of developing acute coronary syndrome. Beyond the growing burden of cardiovascular risk factors among women of childbearing age (17), ageism itself poses a significant threat to women of all racial backgrounds in the United States. However, systematic efforts to address this issue have only emerged in the past decade (18). Conversely, Serbia's annual statistical reports (19) do not provide precise data on differences in non-communicable disease morbidity and mortality rates between men and women in the Roma population, despite their well-documented vulnerability due to social determinants of health (SDOH). Additionally, the ongoing migrant crisis, which began in 2015, is not accounted for – despite *United Nations High Commissioner for Refugees (UNHCR)* reports (20) indicating that Serbia has taken in far more refugees from African countries than initially deemed feasible, even before the influx from Ukraine and Russia in February 2022. Currently, no reports are available on health status of women refugeed in Serbia, whether they are still displaced and live in camps or are in process of integration, although the generous universal coverage makes follow up exams possible for both groups.

Sex-specific risk factors, i.e., adverse pregnancy outcomes, early menopause irrelevant of mechanism, mental health, violence with an emphasis on intimate partner violence, are defined in the last State-of-the-Art Review for Primary Prevention of Cardiovascular Disease in Women (21). However, the window of opportunity – often referred to as the "fourth trimester" (13) – remains largely overlooked, not only among African American women but across populations more broadly (22). Fortunately, awareness of this critical period is increasing, as long-term health risks are now being recognized globally, including in Scandinavia (23).

The emergence of the SARS-CoV-2 virus and the COVID-19 pandemic exposed yet another layer of suboptimal care for women. Initially, misinformation suggesting that women were less susceptible to infection led to their disproportionate placement on the frontlines, increasing their exposure to patients more than men (24). This was followed by numerous reports highlighting higher mortality rates, more complex clinical presentations, and increased morbidity among women. Finally, the lingering effects of *long COVID*, along with its cardiovascular complications and the confounding impact of vaccines, have further underscored these disparities (9, 25-29). All of the above has not only driven the need for more sex-specific research designs but also highlighted the importance of addressing the infodemic and the role of health literacy in its lingering detrimental effects.

Aims

Given the limited national data on this topic, we aimed to assess not only hospitalization rates among women and men but also their awareness of risk factors for both themselves and the opposite sex. Additionally, we sought to explore preferred methods of communication with the healthcare system and their willingness to engage with telemedicine options.

METHODS

Our prospective research protocol was conducted at the Division of Cardiology of the University Clinical Center of Serbia from January 1st, 2024 to February 24th, 2024 with the ethical approval obtained from the Institutional Review Board of the Faculty of Medicine, University of Belgrade (December 2023).

During the specified timeframe, all patients hospitalized in two departments of our Division were systematically invited to participate in an educational interview and complete a multiple-choice questionnaire (MCQ). The questionnaire focused on patients' demographics, the presence and awareness of risk factors, preferred communication modalities for follow-up, and interest in telemedicine options. All patients signed an informed consent and provided contact information in case they wished to pursue follow up or refer family members they identified as unaware of cardiovascular burden.

The limitations of this pilot study included several factors: variations in local academic deadlines tied to the research protocol, which resulted in a two-week shortening of the study timeline;the holiday season, which added five days of complexity to patient inclusion, as well as the 14 weekend days; PCR positivity results for seasonal infections (COVID-19, Influenza A, Influenza B) led to mandatory immediate discharge by the hospital's infection control protocols, requiring follow-up and treatment at secondary or primary healthcare levels (as a result, patients not already included could not be retained for same-day participation or discharge); patients who underwent coronary angiography through the Day Hospital often stayed less than 12 hours in our department and were less motivated to engage with the local healthcare team providing post-catheterization care, resulting in early discharge the following day; denial of participation for various reasons; difficulty in understanding the questions posed; patients' critical condition, dementia, or psychiatric status at the time of screening.

STATISTICAL ANALYSIS

Due to the size, statistical analyses were limited to central tendency measures (mean), dispersion (SD, minimum and maximum), and absolute and relative frequency measurements, with a χ^2 test considered significant at a $p<0.05$ or Fisher's exact test.

RESULTS

Out of a total of 130 patients of both sexes hospitalized between January 1st, 2024 and February 24th, 2024, 49 patients accepted to participate, 66 refused, while 15 had medically and ethically justified reasons for non-inclusion.

Men comprised 51.0% (25) and women 49.0% (24) of the included patients. The average age was 64.24±12.35 years, with the youngest patient being 28 and the oldest 91. The age distribution is shown in **Figure 1**.

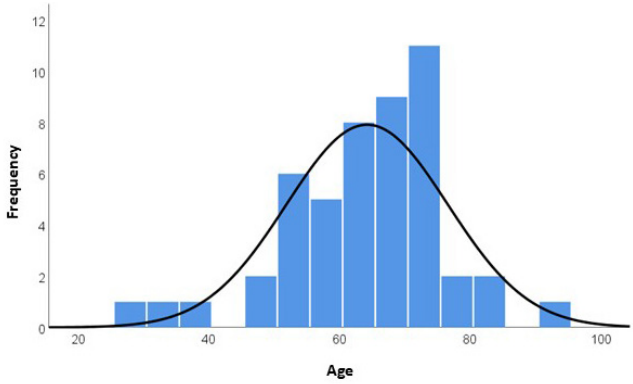

Figure 1. Age distribution of the study population

In regard to risk factors' presence (detailed in **Table 1**) only dyslipidemia was significantly more present in women.

Table 1. Frequency of risk factors according to sex

The presence of risk factors	Sex		*p*-value
	Men (n=25)	Women (n=24)	
Hypertension	18 (72.0)	16 (66.7)	0.686
Type 2 diabetes	10 (40.0)	15 (62.5)	0.115
Dyslipidemia	6 (24.0)	16 (66.7)	**0.003**
Obesity	12 (48.0)	13 (54.2)	0.666
Smoking	4 (16.0)	4 (16.7)	1.000
Migraine	1 (4.0)	5 (20.8)	0.098
Prior cancer treatment	1 (4.0)	5 (20.8)	0.098
Burnout	8 (32.0)	12 (50.0)	0.200
Physical activity	12 (48.0)	11 (45.8)	0.879
Alcohol	6 (24.0)	1 (4.2)	0.098

Data were presented as n (%)

On the other hand, awareness levels for traditional and modern risk factors were low in both sexes and with no significant difference between the two groups, as show in **Table 2.**

Phone calls and text messages were the preferred modes of contact with the healthcare team, with no statistically significant difference between the sexes. As expected, women primarily served as household caregivers for the included men **(Table 3)**.

Table 2. Frequency of risk factors' awareness according to sex

The presence of risk factors	Sex		*p*-value
	Men (n=25)	Women (n=24)	
Hypertension	23 (92.0)	23 (95.8)	1.000
Type 2 diabetes	22 (88.0)	22 (91.7)	1.000
Dyslipidemia	20 (80.0)	20 (83.3)	1.000
Obesity	21 (84.0)	24 (100.0)	0.110
Smoking	23 (92.0)	21 (87.5)	0.667
Migraine	22 (88.0)	22 (91.7)	1.000
Family history	9 (36.0)	10 (41.7)	0.684
Early menarche	0 (0.0)	4 (16.7)	1.000
Late menarche	0 (0.0)	3 (12.5)	1.000
Infertility	0 (0.0)	3 (12.5)	1.000
In vitro fertilization	0 (0.0)	5 (20.8)	1.000
Adverse pregnancy outcomes	0 (0.0)	5 (20.8)	1.000
Peripartum complications	0 (0.0)	6 (25.0)	1.000
Prior cancer treatment	13 (68.4)	13 (54.2)	0.342
Burnout	20 (80.0)	14 (58.3)	0.100

Data were presented as n (%)

Table 3. Frequency of preferential contact with the healthcare team and follow up according to sex

Telemedicine options	Sex		*p*-value
	Men (n=25)	Women (n=24)	
Phone call	19 (76,0)	18 (75,0)	1,000
Text message	15 (60,0)	11 (45,8)	0,321
Apps	6 (24,0)	7 (29,2)	0,682
Email	7 (28,0)	9 (37,5)	0,478
Social media engagement	3 (12,0)	3 (12,5)	1,000
Telemedicine, in general	1 (4,0)	2 (8,3)	0,609
Choice of healthcare team member for telemedicine	1 (4,2)	2 (8,7)	0,609
Societal support			
No one/Almost no one	12 (48,0)	16 (66,7)	0,187
Average/Maximum Support	13 (52,0)	8 (33,3)	

Data were presented as n (%)

DISCUSSION

In our pilot study sample, we observed a lower hospitalization rate among women, who were older and had more comorbidities than men. These findings align with global data indicating a lower level of awareness regarding sex-specific risk factors and disease presentation, as well as delayed contact with the nearest healthcare service or emergency number, as noted in guidelines from both sides of the Atlantic (30-32). Despite existing discrimination in the diagnostic and treatment approaches for conditions like MINOCA/INOCA, SCAD, and myocardial bridges (9, 16, 33-37), recent reports confirming a two-fold higher mortality in patients presenting with MINOCA during acute coronary syndrome (38) may help reduce the stigma surrounding these conditions, even in the face of ongoing appeals (14, 16, 33, 35) and the challenges posed by the COVID-19 pandemic on interventional cardiology services(24, 39, 40).

Regrettably, when comparing the health literacy levels on both traditional and modern risk factors for the development and progression of cardiovascular disease in our unit's post-pandemic setting, the awareness levels were not significantly improved compared to the previous year (41). However, women – particularly those over 60 years of age – were more informed about the link between reproductive and cardiovascular health, recognizing that reproductive history serves as a window into later cardiovascular and cerebrovascular risks, morbidity, and mortality. These connections are well-established (7, 21, 42-44), but it is up to all stakeholders and policymakers to raise societal awareness about cardiovascular health beyond the reproductive health focus, which has been traditionally promoted through the "bikini medicine" approach (9, 15, 45), to ultimately improve long-term cardiovascular health (45, 46).

All modern risk factors were poorly recognized, highlighting a pervasive low level of health literacy that appears to be thriving in the era of the COVID-19 infodemic, despite well-established medical (47-53) and obstetric (12, 49, 54-57) knowledge on conditions that increase long-term cardiovascular risk in pregnant individuals – a term still absent from the Serbian medical lexicon, despite recommendations from international medical societies dedicated to gynecology and obstetrics. This gap in understanding also extends to the offspring, regardless of the method of conception.

What was surprising was the high level of resistance encountered when attempting to engage in educational conversations with men, particularly those who showed little interest in understanding or addressing their own risk factors. None of these individuals took the opportunity to schedule follow-up exams for themselves or for family members they recognized as having suboptimally controlled risk factors, across any level of prevention – from primordial to quaternary. Additionally, an interesting observation was made by two mid-career male team members: hospitalized men exhibited a culturally typical familiarity that bordered on rudeness in their communication with the healthcare team, particularly in interactions with junior female team members (one fellow, three residents, and one student). However, they tended to avoid engaging in similar behavior with mid-career female professionals (three attendings). This pattern was later confirmed by nursing staff. The described hostility, which could be interpreted as misogyny, was not quantified and could be considered a secondary outcome. This was not anticipated initially, and the research protocol did not include a specific questionnaire to capture such experiences among the various existing ones. Additionally, a comparison of these experiences with those from international studies in different settings was not deliberately made to avoid potential bias. A larger, more targeted study on this issue should be conducted in future.

In contrast to the denialists, men who agreed to participate in completing the MCQ reported female household members as their primary source of social support. This aligns with a global phenomenon, irrespective of cultural

context (24, 39), where women are often responsible for well-established forms of unpaid work, a situation further exacerbated by the COVID-19 pandemic (24, 39, 58).

Although telemedicine implementation in Serbia was derailed by COVID19, modest national attempts in the domain of cardiovascular health of women have been made (59, 60), so our seeking to understand better the needs of the patients of both sexes led us to conducting the MCQ part on willingness to embrace new technologies that are still not used in medical practice regionally. In our current sample, the motivation to use various existing technologies – ranging from text messages and phone calls to health-focused mobile apps and social media – was low for both women and men. While no statistical significance was found, this motivation was notably higher in women just one year ago (41). These results are not surprising, as previous studies have shown that university students in France, in general, use health-related websites and apps (61). However, our own study of final-year medical and sports medicine students (62) revealed that sex-specifically being female – was a predictor for using health apps, but only for the sports medicine group, not the medical students. Alarmingly, only 30% of female medical students reported using menstrual apps, considered helpful for reproductive health, compared to 41.4% of female sports medicine students. The national results, gathered within a similar timeframe at Belgrade University, underscore the critical importance of women's heart centers and programs (9-11), such as the one launched at the University Clinical Center of Serbia (9, 63). These centers provide interdisciplinary and multidisciplinary diagnostic services, along with timely treatment for women throughout their lifespan, aiming to protect the most vulnerable populations. Additionally, they play a crucial role in optimizing health literacy across all social strata, with a focus on local logistical considerations (9, 16, 64-66). These results highlight the need to modernize the core curriculum, extending beyond workshops for biomedicine students (67) to include tailored sessions for general practitioners (59) and cardiologists (60, 68-70). Modest efforts have already been made, such as the introduction of the concept of preventive measures across a woman's lifespan in the cardiology fellowship program since 2023, included in one of the Belgrade University Faculty of Medicine's "Q & A" manuals (71). Furthermore, from a public health perspective, where low health literacy impacts expenditures within our still-solidarity-based universal coverage health insurance model, further research of this kind could offer valuable educational interviews with patients, while gathering essential data to optimize care standards and necessary metrics, moving us a step closer to improving healthcare outcomes.

CONCLUSION

Despite the numerous limitations of our snapshot pilot research, our findings revealed a modest level of health literacy when patients were questioned about both their own and the opposite sex's cardiovascular disease risk factors. As expected, the social support system predominantly relied on female household and family members. Although not initially planned, researchers – regardless of gender, sex, or age – encountered a level of misogyny that remains to be quantified. Additionally, our results emphasize the importance of leveraging telemedicine and social media as sources of healthcare information, particularly for women, who showed a higher interest in using handheld devices to improve their own health and that of their family members.

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Conflicts of interest

None to declare pertaining to the conducted pilot study

Author contributions

Conception or design of the work: BP, NKW, ZL, SDjD, DS, VK.
Acquisition: ZG, OB, MDj, PS, JVB, SP, DDj, IPDj, BP.
Analysis and interpretation of data: AMM, BP.
Preparing the draft of the manuscript: BP, PS, AMM, MDj.

Ethical approval

Faculty of Medicine University of Belgrade IRB approval (Dec 2023)

REFERENCE

1. Martin SS, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, et al. 2024 Heart Disease and Stroke Statistics: A Report of US and Global Data From the American Heart Association. Circulation. 2024;149(8):e347-e913.
2. Lloyd-Jones DM, Allen NB, Anderson CAM, Black T, Brewer LC, Foraker RE, et al. Life's Essential 8: Updating and Enhancing the American Heart Association's Construct of Cardiovascular Health: A Presidential Advisory From the American Heart Association. Circulation. 2022;146(5):e18-e43.
3. Parapid B. Metabolic Syndrome and Cardiovascular Morbidity and Mortality In the National Cohorts of The Seven Countries Study - The 40 Years' Follow Up Results. Belgrade, Serbia: Belgrade University - Faculty of Medicine; 2014.

4. Shan Z, Li Y, Zong G, Guo Y, Li J, Manson JE, et al. Rotating night shift work and adherence to unhealthy lifestyle in predicting risk of type 2 diabetes: results from two large US cohorts of female nurses. Bmj. 2018;363:k4641.
5. Fujishiro K, Lividoti Hibert E, Schernhammer E, Rich-Edwards JW. Shift work, job strain and changes in the body mass index among women: a prospective study. Occup Environ Med. 2017;74(6):410-6.
6. Shi H, Huang T, Schernhammer ES, Sun Q, Wang M. Rotating Night Shift Work and Healthy Aging After 24 Years of Follow-up in the Nurses' Health Study. JAMA Netw Open. 2022;5(5):e2210450.
7. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140(11):e596-e646.
8. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice: Developed by the Task Force for cardiovascular disease prevention in clinical practice with representatives of the European Society of Cardiology and 12 medical societies With the special contribution of the European Association of Preventive Cardiology (EAPC). European Heart Journal. 2021;42(34):3227-337.
9. Parapid B, Kanjuh V, Kostić V, Polovina S, Dinić M, Lončar Z, et al. Women's Health in Serbia - Past, Present, and Future. Srpski Arhiv za Celokupno Lekarstvo. 2021;149(11-12):745-54.
10. Lundberg GP, Mehta LS, Sanghani RM, Patel HN, Aggarwal NR, Aggarwal NT, et al. Heart Centers for Women: Historical Perspective on Formation and Future Strategies to Reduce Cardiovascular Disease. Circulation. 2018;138(11):1155-65.
11. Gulati M, Hendry C, Parapid B, Mulvagh SL. Why We Need Specialised Centres for Women's Hearts: Changing the Face of Cardiovascular Care for Women. Eur Cardiol. 2021;16:e52.
12. Bond RM, Phillips K, Ivy KN, Ogueri V, Parapid B, Miller SC, et al. Cardiovascular Health of Black Women Before, During, and After Pregnancy: A Call to Action and Implications for Prevention. Current Cardiovascular Risk Reports. 2022.
13. Bond RM, Gaither K, Nasser SA, Albert MA, Ferdinand KC, Njoroge JN, et al. Working Agenda for Black Mothers: A Position Paper From the Association of Black Cardiologists on Solutions to Improving Black Maternal Health. Circ Cardiovasc Qual Outcomes. 2021;14(2):e007643.
14. Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, et al. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. Lancet. 2021;397(10292):2385-438.
15. Wenger NK. The Feminine Face of Heart Disease 2024. Circulation. 2024;149(7):489-91.
16. Manzo-Silberman S, Hawranek M, Banerjee S, Kaluzna-Oleksy M, Alasnag M, Paradies V, et al. Call to action for acute myocardial infarction in women: international multi-disciplinary practical roadmap. Eur Heart J Open. 2024;4(6):oeae087.
17. Manzo-Silberman S, Chabbert-Buffet N, Roux E, Parisi M, Regidor PA, Mounier-Vehier C. Prevalence of cumulative cardiovascular risk factors among women of childbearing age in France: Results of the GYNRISK® survey. J Gynecol Obstet Hum Reprod. 2024;54(1):102859.
18. Alexander KP, Rich MW, Forman DE, Wenger NK, Dodson JA, Alpert JS, et al. Top 10 List for the Cardiovascular Care of Older Adults. Am J Med. 2016;129(9):901-2.
19. INSTITUTE OF PUBLIC HEALTH OF SERBIA DMJB. HEALTH STATISTICAL YEARBOOK OF REPUBLIC OF SERBIA 2022. Beograd, Serbia.; 2023.
20. UNHCR. The Balkan Migrant Crisis 2016 [Available from: https://data2.unhcr.org/en/documents/download/46801.
21. Cho L, Davis M, Elgendy I, Epps K, Lindley KJ, Mehta PK, et al. Summary of Updated Recommendations for Primary Prevention of Cardiovascular Disease in Women: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(20):2602-18.
22. Lewey J, Beckie TM, Brown HL, Brown SD, Garovic VD, Khan SS, et al. Opportunities in the Postpartum Period to Reduce Cardiovascular Disease Risk After Adverse Pregnancy Outcomes: A Scientific Statement From the American Heart Association. Circulation. 2024;149(7):e330-e46.
23. Kvalvik LG, Skjærven R, Sulo G, Singh A, Harmon QE, Wilcox AJ. Pregnancy History at 40 Years of Age as a Marker of Cardiovascular Risk. Journal of the American Heart Association.0(0):e030560.
24. Parapid B, Alasnag M, Hayes S, Samargandy S, Banerjee S, Alasnag M, et al. COVID-19 impact on women on both sides of the frontline - the American College of Cardiology Women in Cardiology Section's International Working Group perspective. Srpski Arhiv Za Celokupno Lekarstvo. 2020;148(9-10):637-43.
25. Parapid B, Bond RM. COVID-19: An Insult to Injury on Equity. International Journal of Cardiovascular Sciences. 2021;34(4):342-3.
26. Sakurada Y, Matsuda Y, Motohashi K, Hasegawa T, Otsuka Y, Nakano Y, et al. Clinical characteristics of female long COVID patients with menstrual symptoms: a retrospective study from a Japanese outpatient clinic. J Psychosom Obstet Gynaecol. 2024;45(1):2305899.
27. Maybin JA, Watters M, Rowley B, Walker CA, Sharp GC, Alvergne A. COVID-19 and abnormal uterine bleeding: potential associations and mechanisms. Clin Sci (Lond). 2024;138(4):153-71.
28. Julio S, Takehiro T, Jamie W, Peiwen L, Sasha T, Jeffrey G, et al. Sex differences in symptomatology and immune profiles of Long COVID. medRxiv. 2024:2024.02.29.24303568.
29. Thaweethai T, Jolley SE, Karlson EW, Levitan EB, Levy B, McComsey GA, et al. Development of a Definition of Postacute Sequelae of SARS-CoV-2 Infection. Jama. 2023;329(22):1934-46.
30. Blankstein R, Gulati M, Jaber WA, Bullock-Palmer RP, Bhatt DL, Shaw LJ. The 2021 Chest Pain Guideline: A Revolutionary New Paradigm for Cardiac Testing. JACC Cardiovasc Imaging. 2022;15(1):140-4.
31. Byrne RA, Rossello X, Coughlan JJ, Barbato E, Berry C, Chieffo A, et al. 2023 ESC Guidelines for the management of acute coronary syndromes: Developed by the task force on the management of acute coronary syndromes of the European Society of Cardiology (ESC). European Heart Journal. 2023;44(38):3720-826.
32. Vrints C, Andreotti F, Koskinas KC, Rossello X, Adamo M, Ainslie J, et al. 2024 ESC Guidelines for the management of chronic coronary syndromes. Eur Heart J. 2024.
33. Alasnag M, Jelani Q-u-a, Johnson TW, Parapid B, Balghaith M, Al-Shaibi K. The Role of Imaging for MINOCA (Myocardial Infarction with No Obstructive Coronary Artery Disease): a Review of Literature and Current Perspectives. Current Cardiovascular Imaging Reports. 2020;13(7):21.
34. Alasnag M, Manzo-Silberman S. Women making waves Editorial: Impact of sex in the functional assessment of intermediate coronary lesions by instantaneous wave-free ratio. Cardiovasc Revasc Med. 2022.
35. Kunadian V, Chieffo A, Camici PG, Berry C, Escaned J, Maas A, et al. An EAPCI Expert Consensus Document on Ischaemia with Non-Obstructive Coronary Arteries in Collaboration with European Society of Cardiology Working Group on Coronary Pathophysiology & Microcirculation Endorsed by Coronary Vasomotor Disorders International Study Group. EuroIntervention. 2021;16(13):1049-69.
36. Donataccio MP, Puymirat E, Parapid B, Steg PG, Eltchaninoff H, Weber S, et al. In-hospital outcomes and long-term mortality according to sex and management strategy in acute myocardial infarction. Insights from the French ST-elevation and non-ST-elevation Myocardial Infarction (FAST-MI) 2005 Registry. Int J Cardiol. 2015;201:265-70.
37. Aleksandric S, Djordjevic-Dikic A, Beleslin B, Parapid B, Teofilovski-Parapid G, Stepanovic J, et al. Noninvasive assessment of myocardial bridging by coronary flow velocity reserve with transthoracic Doppler echocardiography: vasodilator vs. inotropic stimulation. Int J Cardiol. 2016;225:37-45.
38. Quesada O, Yildiz M, Henry TD, Bergstedt S, Chambers J, Shah A, et al. Mortality in ST-Segment Elevation Myocardial Infarction With Nonobstructive Coronary Arteries and Mimickers. JAMA Network Open. 2023;6(11):e2343402-e.

39. Öz TK, Cader FA, Dakhil ZA, Parapid B, Kadavath S, Bond R, et al. International consensus statement on challenges for women in cardiovascular practice and research in the COVID-19 era. Minerva Cardiol Angiol. 2022;70(6):641-51.
40. De Luca G, Manzo-Silberman S, Algowhary M, Uguz B, Oliveira DC, Ganyukov V, et al. Gender Difference in the Effects of COVID-19 Pandemic on Mechanical Reperfusion and 30-Day Mortality for STEMI: Results of the ISACS-STEMI COVID-19 Registry. J Clin Med. 2023;12(3).
41. Djapanović VP, A., editor Risk Factors for the Development of Early Cardiovascular Disease in Women: Results of the University Clinical Center of Serbia Pilot Study. 62 Kongres Studenata Biomedicinskih Nauka sa Internacionalnim Učešćem; 2023; Kopaonik, Srbija 24-28.04.2023. Belgrade, Serbia: Medicinski fakultet Univerziteta u Beogradu; 2023.
42. Bilen O, Wenger NK. Hypertension management in older adults. F1000Res. 2020;9.
43. Faulkner JL. Obesity-associated cardiovascular risk in women: hypertension and heart failure. Clin Sci (Lond). 2021;135(12):1523-44.
44. Flouris AD, Faught BE, Klentrou P. Cardiovascular disease risk in adolescent smokers: evidence of a 'smoker lifestyle'. J Child Health Care. 2008;12(3):221-31.
45. Wenger NK, Lorell BH, Bairey Merz CN. Recent Legislation, Public Policy Changes, and Women's Cardiovascular Health: Disproportionate Burden. Circulation. 2018;138(11):1082-4.
46. Kouvari M, Souliotis K, Yannakoulia M, Panagiotakos DB. Cardiovascular Diseases in Women: Policies and Practices Around the Globe to Achieve Gender Equity in Cardiac Health. Risk Manag Healthc Policy. 2020;13:2079-94.
47. Lee I, Vresilovic J, Irfan M, Gallop R, Dokras A. Higher Incidence of Metabolic Syndrome in Black Women With Polycystic Ovary Syndrome: A Longitudinal Study. J Clin Endocrinol Metab. 2021.
48. Woodward A, Klonizakis M, Lahart I, Carter A, Dalton C, Metwally M, et al. The effects of exercise on cardiometabolic outcomes in women with polycystic ovary syndrome not taking the oral contraceptive pill: protocol for a systematic review and meta-analysis. Syst Rev. 2019;8(1):116.
49. Gojnic M, Todorovic J, Stanisavljevic D, Jotic A, Lukic L, Milicic T, et al. Maternal and Fetal Outcomes among Pregnant Women with Diabetes. Int J Environ Res Public Health. 2022;19(6).
50. Elder P, Sharma G, Gulati M, Michos ED. Identification of female-specific risk enhancers throughout the lifespan of women to improve cardiovascular disease prevention. American Journal of Preventive Cardiology. 2020;2:100028.
51. Agarwala A, Michos ED, Samad Z, Ballantyne CM, Virani SS. The Use of Sex-Specific Factors in the Assessment of Women's Cardiovascular Risk. Circulation. 2020;141(7):592-9.
52. Osibogun O, Ogunmoroti O, Michos ED. Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention. Trends Cardiovasc Med. 2020;30(7):399-404.
53. Parapid B, Rakić S. Oral Contraception: Beyond What Meets the Eye. Sorry, the Ovaries! International Journal of Cardiovascular Sciences. 2022;35(4):511-3.
54. Debbink MP, Ugwu LG, Grobman WA, Reddy UM, Tita ATN, El-Sayed YY, et al. Racial and Ethnic Inequities in Cesarean Birth and Maternal Morbidity in a Low-Risk, Nulliparous Cohort. Obstet Gynecol. 2022;139(1):73-82.
55. Catov JM, McNeil RB, Marsh DJ, Mercer BM, Bairey Merz CN, Parker CB, et al. Early Pregnancy Atherogenic Profile in a First Pregnancy and Hypertension Risk 2 to 7 Years After Delivery. J Am Heart Assoc. 2021;10(5):e017216.
56. Kuo CH, Kuo CC, Wu HY, Wu DC, Yang CY. Higher parity and earlier age at first birth are associated with lower risk of death from colon cancer. Cancer Sci. 2012;103(8):1553-7.
57. Sharma G, Zakaria S, Michos ED, Bhatt AB, Lundberg GP, Florio KL, et al. Improving Cardiovascular Workforce Competencies in Cardio-Obstetrics: Current Challenges and Future Directions. J Am Heart Assoc. 2020;9(12):e015569.
58. UN_WOMEN. Facts and figures: Economic empowerment 2024 [Available from: https://www.unwomen.org/en/what-we-do/economic-empowerment/facts-and-figures.
59. Parapid B, editor Kardiovaskularno zdravlje žena. 11Kongres Lekara Opšte Medicine Srbije, pod okriljem Sekcije Opšte Medicine SLD; 2021 24.09.2021.; Zlatibor, Srbija.
60. Parapid BL, B., editor Žensko kardiovaskularno zdravlje – prikaz rezultata. . 21 Kongres Udruženja Kardiologa Srbije 2021 22.10.2021.; Beograd, Srbija.
61. Montagni I, Cariou T, Feuillet T, Langlois E, Tzourio C. Exploring Digital Health Use and Opinions of University Students: Field Survey Study. JMIR Mhealth Uhealth. 2018;6(3):e65.
62. Gazibara TC, M.; Cakić, J.; Zejnelagić, R.; Popović, A.; Grgurević, A. Factors associated with use of health websites and apps among students in medicine and sports. Medicinska istraživanja. 2023;57(1):83-91.
63. A. DVP, editor Risk Factors for the Development of Early Cardiovascular Disease in Women: Results of the University Clinical Center of Serbia Pilot Study. 62 Kongres Studenata Biomedicinskih Nauka sa Internacionalnim Učešćem; 2023; Kopaonik, Srbija 24-28.04.2023. 2023: Medicinski fakultet Univerziteta u Beogradu.
64. Wenger NK, Williams OO, Parashar S. SMARTWOMAN™: Feasibility assessment of a smartphone app to control cardiovascular risk factors in vulnerable diabetic women. Clin Cardiol. 2019;42(2):217-21.
65. Krishnaswami A, Beavers C, Dorsch MP, Dodson JA, Masterson Creber R, Kitsiou S, et al. Gerotechnology for Older Adults With Cardiovascular Diseases: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;76(22):2650-70.
66. Schorr EN, Gepner AD, Dolansky MA, Forman DE, Park LG, Petersen KS, et al. Harnessing Mobile Health Technology for Secondary Cardiovascular Disease Prevention in Older Adults: A Scientific Statement From the American Heart Association. Circ Cardiovasc Qual Outcomes. 2021;14(5):e000103.
67. Parapid B, editor Žensko kardiovaskularno zdravlje. 21 Kongres Studenata Biomedicine; 2022 25-29.04.2022.; Kopaonik, Srbija.
68. Parapid B, editor Gender dispariates and coronary artery disease: Women's health. in Expert lectures 5. BASICS+ 7th (Belgrade Summit of Interventional Cardiologists); 2011 April 8-9, 2011; Belgrade, Serbia.
69. Parapid B, editor Women's Heart Programs: A Bare Necessity of Diversity & Inclusion, not a Fashion Accessory of Innovation NOVAK 2022 - Symposium of the Cardiology society of Serbia - Clinical update and perspectives of cardiology in Serbia; 2022 28-30.10.2022.; Novi Sad, Serbia.
70. Parapid B, editor ESC 2023: innovations in the treatment of cardiovascular diseases in women or not so much? Serbia Prevent 2023; 2023 28-29.09.2023.; Belgrade, Serbia.
71. Parapid B. LK, Gojnić-Dugalić M., Kanjuh V.I., Kass Wenger, N. Koje su specifičnosti KV prevencije kod žena? In: Beleslin B, editor. Cardiologia preventiva: kroz 120 pitanja i odgovora. Beograd.: Data status.; 2023.

SVEST O POLNO SPECIFIČNIM FAKTORIMA RIZIKA, PRIKRIVENA MIZOGINIJA I DUGOROČNA OPTIMIZACIJA KARDIOVASKULARNOG RIZIKA: REZULTATI PILOT STUDIJE

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Sažetak

UVOD: Iako se zdravstvena pismenost smatra temeljem prevencije, polno specifičnoj prevenciji faktora rizika za razvoj kardiovaskularnih bolesti, vodećem uzroku mortaliteta žena globalno, i dalje se ne pridaje dovoljno značaja, a ustaljena kulturološka uverenja prednjače u odnosu na zvanične preporuke.

METODE: Validirani upitnik kreiran kako bi edukovao bolesnika, a sa druge strane pomogao zdravstvenom timu da uči o upravljanju tradicionalnim i polno specifičnim modernim faktorima rizika, uključujući mogućnost sveobuhvatnog individualnog dugoročnog praćenja, ponuđen je svim hospitalizovanim pacijentima.

REZULTATI: Od 130 pacijenata hospitalizovanih na odeljenju u periodu od 01.01. do 24.02.2024. kojima je upitnik ponuđen, 37.7% prihvatilo je da učestvuje, dok je 11.5% bilo isključeno iz različitih etičkih razloga. Ispitivane žene (49%) bile su starije od muškaraca, imale su viši stepen opterećenja tradicionalnim faktorima rizika, ali su posedovale i viši stepen znanja i zainteresovanosti za faktore rizika kod oba pola, dok su muškarci, posebno oni koji su odbili da učestvuju u anketiranju iz nepoznatog razloga, pokazali nezainteresovanost za navedene faktore rizika čak i kod bliskih rođaka i ukućanki. Muškarci za razliku od žena nisu pokazali zainteresovanost za upotrebu savremenih modaliteta telemedicine, iako moderna sredstva komunikacije koriste u gotovo istoj meri kao žene.

ZAKLJUČAK: Dobijeni rezultati u skladu su sa onim dobijenim tokom pilot istraživanja sprovedenog samo na ženama prethodne godine. Međutim, ono što iznenađuje kada su u pitanju muški ispitanici jeste hostilnost iskazana prema kako mlađim, tako i doktorkama srednje generacije, koja nestaje kada im se obrati muškarac starije generacije kao član zdravstvenog tima. Ovakav rezultat potvrđuje da je mizoginiju potrebno aktivno suzbijati.

Ključne reči: zdravstvena pismenost, kardiovaskularna bolest, kardiovaskularni rizik, polno specifični faktori rizika, mizoginija

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ORIGINAL ARTICLE

The mental well-being of medical students: do lifestyles and physical activity make any difference?

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Summary

Introduction: Lifestyle—including dietary habits, physical activity, smoking, and alcohol consumption—plays a crucial role in shaping both individual and population health. Medical students often have a suboptimal activity level and unhealthy lifestyle habits, which affect their well-being and future medical practice. The aim of this research was to examine the frequency of insufficient physical activity and lifestyle factors associated with it among fifth-year medical students at the Faculty of Medicine, University of Belgrade, as well as their association with the physical and mental health of students.

Methods: A cross-sectional study was conducted among fifth-year medical students at the Faculty of Medicine, University of Belgrade, during the social medicine course in November 2023. An anonymous questionnaire was used to assess physical activity, lifestyle characteristics, and symptoms of depression and anxiety. The study included 432 students, achieving a 90.4% participation rate. Based on energy expenditure, participants were categorized into groups with sufficient and insufficient physical activity levels.

Results: A total of 379 students (92%) belonged to the sufficient physical activity group, while 33 students (8%) were classified as having insufficient physical activity. Multivariate logistic regression exhibited a significant association between physical inactivity and lower BMI (OR: 0.81, 95% CI: 0.69-0.95), more pronounced depression symptoms (OR: 1.07, 95% CI: 1.01-1.15) and poorer financial status (OR: 0.51, 95% CI: 0.27-0.96).

Conclusion: Most of the surveyed students are sufficiently physically active. Physical inactivity was significantly associated with female gender, lower BMI, and more pronounced symptoms of depression.

Keywords: physical activity, lifestyle, obesity, mental health, medical students

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INTRODUCTION

Lifestyle refers to the way of living and social practices adopted by individuals, which reflect personal, group, and socio-economic identities and significantly impact overall physical and mental health (1). The key components of lifestyle include: dietary habits, body weight and composition, physical activity, adequate sleep, and habits related to smoking and alcohol consumption (2, 3). The World Health Organization (WHO) defines physical activity as "any bodily movement produced by skeletal muscles that requires energy expenditure" (4). Physical activity encompasses all movement, including work-related, transport, or leisure-time activities, and can involve walking, cycling, sports, active recreation, and play (5, 6). According to WHO guidelines, individuals aged 18–64 are advised to engage in 150–300 minutes of moderate-intensity aerobic physical activity or 75–150 minutes of vigorous-intensity aerobic physical activity per week. Additional health benefits could be achieved by doing more than 300 or more than 150 minutes of moderate-intensity aerobic or high-intensity activity, respectively. Additionally, engaging in muscle-strengthening activities targeting all major muscle groups at least twice weekly provides further health benefits (7).

Adopting a healthy lifestyle and habits, along with regular physical activity, has significant health benefits, reduces the risk of chronic non-communicable diseases, which are currently the leading cause of morbidity and mortality worldwide, and alleviates economic costs related to both individual healthcare and the healthcare system (8, 9). Regular physical activity facilitates healthy growth and development, especially in young people, and also reduces symptoms of depression and anxiety, and improves mood, cognitive processes, learning, judgment, and sleep quality (5, 10, 11). A healthy lifestyle is fundamental for overall health and well-being and appropriate cognitive and social development in young adults. Many factors have been identified as potential predictors of a healthy lifestyle and sufficient physical activity, including male gender, higher socio-economic status, living in an urban environment, higher education level, and higher academic performance (12, 13).

In the context of lifestyle and physical activity research, medical students are a distinct group of interest (12). Firstly, they are considered particularly vulnerable due to their new responsibilities, challenges, lack of free time, and academic stress, all associated with enrolling and studying at the University. Upon entering medical school, there is a tendency for changes in personal dietary and other lifestyle habits, and students often face the challenge of balancing academic, personal, and social lives (10-12). Secondly, lifestyle habits are typically established early in life and, once formed, can be challenging to change, often persisting over time. These habits influence personal health and can impact future careers and patient well-being (3, 14). Thirdly, medical students are future physicians and healthcare providers who will be a significant part of the healthcare system (15). Physicians with healthy habits, regular physical activity, and an overall healthy lifestyle serve as role models and provide ongoing health education and promotion of healthy lifestyles to patients and the general population (10). Previous research suggests that physicians with healthy habits are more likely to counsel patients on diet, physical activity, and lifestyle. Unfortunately, there is a lack of sufficient data and research concerning medical students' lifestyles and physical activity (10, 16).

The general belief is that medical students, compared to other students, have greater interest and knowledge about various aspects of health, healthy living habits, and their benefits. However, the use of this theoretical knowledge in personal life is not actively encouraged, and in some cases, it is even discouraged due to the large amount of time medical students invest in their studies (3, 14). The level of physical activity among students is generally suboptimal, which is prominent among medical students. Results from a global study across 23 countries indicate that physical inactivity among students ranges from 21.9% to 80.6% (7). Other studies have shown that over 70% of students do not meet the recommended daily step count of 10,000 steps (17). Furthermore, a recent study in Poland found that about 40% of medical students are physically inactive (7).

Qualitative studies have provided insight into factors contributing to reduced physical activity among students and they include individual factors (personal discipline, time), social factors (lack of parental control and social support), environmental factors (availability of exercise facilities, cost), and academic factors (exams, obligations) (17).

The aim of this research is to examine the frequency of insufficient physical activity and lifestyle factors associated with physical activity among fifth-year medical students at the Faculty of Medicine, University of Belgrade, as well as the association between physical activity and physical and mental health of medical students.

MATERIAL AND METHODS

The study was conducted as a cross-sectional study among fifth-year students at the Faculty of Medicine, University of Belgrade, during one week of classes in the Social Medicine course in November 2023. All participating students were informed about the purpose of the study and the research methodology and were asked to complete an anonymous questionnaire. Completion and submission of the questionnaire were considered as implied consent to participate in the study. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade (17/X-15). Among the 478 fifth-year students at the Faculty of Medicine, 432

participated in the study, resulting in a response rate of 90.4%. Complete physical activity questionnaires were submitted by 412 students.

The research instrument was a questionnaire developed based on questionnaires used in similar studies, addressing socio-demographic and socio-economic characteristics of the respondents; lifestyle (including physical activity, smoking, cannabis consumption and anxiolytic use); and symptoms of depression and anxiety.

The respondents' physical activity was assessed using the International Physical Activity Questionnaire (IPAQ), which calculates total weekly energy expenditure and classifies individuals into three categories: high, moderate, or low physical activity (18). Based on this, physical activity levels were compared to the WHO recommendations for minimal physical activity.

Energy expenditure = energy expenditure in vigorous physical activity + energy expenditure in moderate physical activity + energy expenditure during walking

*Energy expenditure in vigorous physical activity = 8 * number of days spent in vigorous physical activity * minutes in vigorous physical activity*

*Energy expenditure in moderate physical activity = 4 * number of days spent in moderate physical activity * minutes in moderate physical activity*

*Energy expenditure during walking = 3.3 * number of days spent walking * minutes walking*

Students were categorized into two groups based on energy expenditure according to WHO recommendations for minimal physical activity: the group with insufficient physical activity (energy expenditure below 600 MET-minutes/week) and the group with sufficient physical activity (energy expenditure above 600 MET-minutes/week).

Depressive symptoms were assessed using the PHQ-9 Patient Depression Questionnaire. The Patient Health Questionnaire (PHQ) is a self-administered version of the PRIME-MD diagnostic tool for common mental disorders, while the PHQ-9 is its depression module. Each of the 9 DSM-IV depression criteria is scored as '0' (not at all) to '3' (nearly every day) (19). Anxiety was assessed using the Zung Self-Rating Anxiety Scale. The Zung Self-Rating Anxiety Scale (SAS) is a psychological assessment tool designed to measure the severity of anxiety symptoms in individuals, thus enabling the quantification of a person's anxiety level. It consists of 20 items, which are scored as '1' (none or a little of the time) to '4' (most or all of the time) (20).

Statistical analysis was performed using descriptive and analytical statistical methods. The significance of differences between categorical variables was examined using the chi-square (χ^2) test and Fisher's exact test. The significance of differences between normally distributed numerical variables was examined using the Student's t-test, and the significance of differences between numerical variables without normal distribution was analyzed using the Mann-Whitney U test. The normality of distribution was tested using the Kolmogorov-Smirnov test. Multivariate logistic regression analysis examined the association between insufficient physical activity (outcome variable) and independent variablessuch as gender, BMI, cannabis use, PHQ-9 score and financial status. All analyses were conducted using SPSS for Windows version 22.0.

RESULTS

The study included 412 students, most female (268, or 65%). The average age of respondents was 23.65±1.41 years, and the average academic grade was 8.68±0.89. A total of 369 (89.6%) respondents reported living in an urban area, while the rest lived in a rural area. The majority of students rated their financial status (56.8%), family relationships (79.4%), and personal health (79.6%) as good, while fewer rated them as average, and the smallest proportion rated them as poor. The average BMI (Body Mass Index) was 22.73±3.62. Based on BMI values and WHO recommendations, 288 (69.9%) students were of normal weight, 81 (19.7%) were overweight, 12 (2.9%) were obese, and 30 (7.3%) were underweight. The median energy expenditure was 2586.00 METs. A total of 379 students (92%) met the WHO recommendations for minimal physical activity and were in the sufficient physical activity group, while 33 (8%) were in the insufficient physical activity group.

In the sufficient physical activity category, 36.2% were male compared to 63.8% female, whereas in the insufficient physical activity category, 15.2% were male and 84.8% female, which is statistically significant (p=0.015) **(Table 1)**.

BMI was significantly higher among students with sufficient physical activity, 23.00±3.86, compared to students with insufficient physical activity, 20.84±2.01 (p<0.001) **(Table 1)**.

There were no statistically significant differences between the two categories of students in their average grades. Most students from both categories reported living in urban areas rather than rural ones. Among students with sufficient physical activity, the highest percentage live with their parents, followed by those residing in their apartment, a rented apartment, a student dormitory, or other housing conditions. Students from the category with insufficient physical activity predominantly reside in student dormitories, followed by living with their parents, with equal frequency in their own and rented apartments, and finally, in other conditions. Neither of these parameters was statistically significant. Students from both categories, in terms of financial support, are primarily supported individuals. Therefore, no significant difference was shown. Most students with sufficient physical activity reported having good financial status, while those with insufficient physical activity predominantly rated their financial status as average. In both groups, the fewest

Table 1. Demographic, socioeconomic, physical and mental health characteristics of the respondents and their physical activity

Characteristic	Insufficient physical activity N (%)	Sufficient physical activity N (%)	p-value
Gender			**0.015**
Male	5 (15.2)	136 (36.2)	
Female	28 (84.8)	240 (63.8)	
BMI (X ± SD)	20.84 ± 2.01	23.0 ± 3.86	**<0.001**
Age (X ± SD)	23.67 ± 1.06	23.66 ± 1.41	0.165
Grade average (X±SD)	8.57 ± 0.71	8.69 ± 0.84	0.228
Living area			0.230
Urban	32 (97.0)	337 (89.4)	
Rural	1 (3.0)	40 (10.6)	
Housing			0.385
Own apartment	7 (21.9)	100 (26.5)	
Rented apartment	7 (21.9)	88 (23.3)	
Student dorm	9 (28.1)	55 (14.6)	
Parents house	8 (25.0)	122 (32.3)	
Other	1 (3.1)	13 (3.4)	
Financial support			0.183
Scholarship	1 (3.1)	52 (13.8)	
Supported individual	31 (96.9)	310 (82.0)	
Personal income	0 (0)	15 (4.0)	
Other	0 (0)	1 (0.3)	
Financial status			**0.036**
Poor	3 (9.4)	15 (4.0)	
Average	16 (50.0)	142 (37.6)	
Good	13 (40.6)	221 (58.5)	
Median	2.00	3.00	
Family relations			0.304
Poor	3 (9.1)	20 (5.3)	
Average	6 (18.2)	56 (14.8)	
Good	24 (72.7)	303 (79.9)	
Median	3.00	3.00	
Personal health			0.255
Poor	1 (3.2)	15 (4.1)	
Average	7 (22.6)	48 (13.0)	
Good	23 (74.2)	305 (82.9)	
Median	3.00	3.00	
Cannabis use			**0.045**
Yes	2 (6.1)	75 (20.4)	
No	31 (93.9)	293 (79.6)	
Tobacco products use			0.257
Yes	10 (30.3)	153 (40.4)	
No	23 (69.7)	226 (59.6)	
Anxiolytic use in last 12 months			0.540
Yes	8 (24.2)	111 (29.3)	
No	25 (75.8)	268 (70.7)	
Zung Self-Rating Anxiety scale score ($\overline{X}$ ± SD)	37.80 ± 8.03	34.79 ± 8.83	0.060
PHQ-9 score ($\overline{X}$ ± SD)	5.00 (0 - 27)	4.00 (0 - 25)	**<0.001**

students reported poor financial status. These parameters were statistically significantly different (p=0.360). Perception of personal health status and family relations did not statistically significantly differ between students with sufficient and insufficient physical activity. The most students in both categories rated their family relations and personal health status as good. In contrast, the smallest number described them as poor **(Table 1)**.

Students with sufficient physical activity reported significantly higher cannabis use over the past 12 months, with 20.4% compared to 6.1% in the group with insufficient physical activity (p=0.045). They also had a statistically significantly lower score on the PHQ-9 depression scale, with the median 4.00 compared to 5.00 (p<0.001). Although students with sufficient physical activity reported somewhat more frequent use of tobacco products and anxiolytics in the past 12 months, this difference was not statistically significant. The Zung Self-Rating Anxiety Scale score for students with insufficient physical activity was 37.80±8.03, while for students with sufficient physical activity, it was 34.79±8.83. The difference was not statistically significant **(Table 1)**.

Multivariate logistic regression analysis showed an association between the PHQ-9 depression scale score (OR: 1.07, 95% CI: 1.01-1.15), body mass index (OR: 0.81, 95% CI: 0.69-0.95) and financial status (OR 0.51, 95% CI: 0.27-0.96) with insufficient physical activity **(Table 2)**.

Table 2. Multivariate logistic regression analysis with insufficient physical activity as outcome variable

Characteristic	OR (95% CI)
Gender	
Male	0.73 (0.35 – 2.18)
Female	1.0 reference category
BMI	**0.81 (0.69 – 0.95)**
Cannabis	
Yes	0.36 (0.08 – 1.64)
No	1.0 reference category
PHQ-9 score	**1.07 (1.01 – 1.15)**
Financial status	**0.51 (0.27 - 0.96)**

DISCUSSION

The aim of this study was to examine the frequency of insufficient physical activity, as well as lifestyle and mental health factors associated with it among medical students. The findings revealed that 92% of fifth-year students at the Faculty of Medicine, University of Belgrade, met the WHO recommendations for sufficient physical activity. Although, as univariate models, female gender, poorer financial status, greater use of cannabis, as well as higher BMI and PHQ-9 score were significantly associated with physical inactivity, as multivariate models, only poorer financial status, higher BMI and PHQ-9 score showed significant association.

Given the numerous benefits of physical activity, the finding that only 8% of medical students engage in insufficient physical activity is highly encouraging. Results from other studies on this topic vary. Studies with similar results (7, 8, 13) offer a potential explanation that medical students have better knowledge regarding health, healthy lifestyle, and physical activity and their importance, which leads them to be more focused on their own health (21). Martinović et al. (22) report that more than half of biomedical science students in Split, Croatia have a satisfactory level of physical activity, which can partly be attributed to their desire to achieve a popular physical appearance and aesthetics. In contrast, some studies (23-26) highlight a significantly higher prevalence of physical inactivity and unhealthy eating among medical students, primarily due to lack of time, stress, and demanding schedules (3). Such differences may be attributed to variations in study methodology as well as differences between participants and university environments (10). When evaluating the methodology, it is essential to consider whether physical activity levels were self-reported—an approach that can be subjective and somewhat inaccurate—or measured using a more objective tool. While a large number of studies which used the self-reporting method showed a fairly high percentage of students with sufficient physical activity (7, 8, 13, 21, 22), a study from Nigeria (26) which monitored physical activity levels of each participant using the actigraph accelerometer activity monitor showed fewer promising results, i.e., a lower percentage of students with sufficient physical activity. Another possible explanation for these differences is highlighted in a study from Bahrain (24), which suggests that limited access to exercise facilities at universities may contribute to student inactivity. Additionally, socio-cultural factors, including restrictions imposed by some families on female students' participation in physical activity, further exacerbate inactivity among women. Moreover, a Saudi Arabian study argues that even college location and the nature of studies can contribute to different results. This study found that students who attended College for Emergency Medical Services situated off the main campus were more physically active than their peers who attended the College of Applied Medical Studies of the same University located within the main campus (10).

A statistically significant difference in physical activity was observed between genders, with males being more physically active. In contrast to varying results among studies on physical activity levels among medical students, research on this topic shows consistency with our results. Through a study conducted in Poland (7) it has been shown that female students (77.97%) have higher frequencies of sedentary activities, as well as lower levels of physical activity compared to their male companions. Similarly, another study from India displayed that male students (39.8%) were significantly more active than female students (20.6%) (27). Romero-Blanco et al. (28) suggest that this difference arises from different motivational factors between genders, as well as greater environmental influence on males (social pressure, competition). Generally, men show significantly higher intrinsic motivation based on a desire to achieve mastery, social recognition, strength, and endurance. They are also more motivated by competition and challenge than women are (29). In addition, society's views and expectations regarding traditional male and masculine attributes could also have an impact. One research revealed that, the majority of young Western adult males wish to increase their muscularity levels to attain the perceived social, sexual, and personal benefits associated with the physique (30). On the other hand, in some studies, women expressed that they feel intimidated while exercising in front of others, especially men, as well as that the exercise facilities are often more tailored towards the needs of men, thus making them feel unwelcome (31).

Among students with sufficient physical activity, the average BMI was statistically significantly higher. However, other studies did not show a statistically significant difference or that individuals with lower BMI have higher levels of physical activity (32, 33). Our results could be explained by the assumption that students with higher

BMI may engage in more physical activity in an effort to reduce body weight and achieve their desired physical appearance and optimal health. Additionally, BMI does not account for body composition, so a higher index could also be related to increased muscle mass in physically active students. Therefore, a more precise analysis should include body composition.

Students with sufficient physical activity significantly more often reported good financial status, whereas students with insufficient physical activity more often reported average or poor financial status. These results are consistent with the ones shown in a study conducted by Grujičić et al. (5). A potential explanation for these results is the fact that people with poorer financial status do not possess resources for gyms, adequate sports equipment and sport activities which require financial investments. Moreover, some universities, including the ones of the Western Balkans often do not provide programmes that would enable free sports centers for all students, thus preventing adequate participation of students with poorer financial status in physical activities (5).

In our study, there was no statistically significant difference in terms of type of settlement, housing, financial status, family relations, and average grades between students with sufficient and insufficient physical activity. However, some studies have shown a positive correlation between above-average financial status, urban area, good family relations, support, higher grades, and higher levels of physical activity (11, 34).

Consistent with the well-documented positive effects of physical activity on mental health and depression (35–38), our study found that students with sufficient physical activity had lower PHQ-9 scores, indicating fewer symptoms of depression compared to those with insufficient activity. Sloan et al. (39) consider physical activity a significant protective factor against depression, poor sleep quality, and psychopathological symptoms. This claim is also supported by population-based observational studies, which have shown that physically active people were nearly 45% less likely to suffer from depressive symptoms than inactive people (40). Given these results, it is clear how important an active lifestyle is throughout life, especially during the vulnerable period of college education (11). Although there is evidence that physical inactivity increases the likelihood of anxiety by 1.75-2 times, anxiety did not show a statistically significant difference in our study (40).

Although cannabis use was higher among students with sufficient physical activity, there was no statistically significant association in multivariate logistic regression. Additionally, there was no statistically significant difference in tobacco use and anxiolytic use.

This study had several limitations. Primarily, it was a cross-sectional study, and therefore causal relationships between parameters cannot be inferred. The research instrument was a self-reported questionnaire, which introduces potential subjectivity and varying interpretations of certain questions. Also, participants were students from one year at one faculty, and there was a large difference in the size of the physically active and inactive groups, so the results cannot be generalized. Given these considerations, future studies should incorporate more objective measures of physical activity, such as sports watches, step counters, and physical fitness tests. Additionally, including students from different years of study and various universities could help identify factors that promote or hinder physical activity. Longitudinal research following a cohort of students from their first to final year of study would also provide valuable insights into changes in physical activity over time.

CONCLUSION

The majority of fifth-year students at the Faculty of Medicine in Belgrade have a sufficient level of physical activity. In this sample, physical inactivity was significantly associated with lower BMI, more pronounced symptoms of depression and poorer financial status. Therefore, it is important to emphasize the role of physical activity as a protective factor against depression. Bearing in mind the positive impact of physical activity on physical, mental and social well-being, we should strive to create an environment which will further enable and encourage physical activity and healthy lifestyle habits among students, especially medical students, as they represent one of the key pillars of our healthcare system.

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Conflicts of interest: None to declare.

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REFERENCES

1. LIFESTYLE DISEASES: An Economic Burden on the Health Services | United Nations. (n.d.). United Nations. Available from: https://www.un.org/en/chronicle/article/lifestyle-diseases- economic-burden-health-services
2. Azami Gilan B, Janatolmakan M, Ashtarian H, Rezaei M, Khatony A. Health-Promoting Lifestyle and Associated Factors among Medical Sciences Students in Kermanshah, Iran: A Cross-Sectional Study. J Environ Public Health. 2021 Apr 26; 2021:6691593.

3. Bin Abdulrahman KA, Khalaf AM, Bin Abbas FB, Alanezi OT. The Lifestyle of Saudi Medical Students. Int J Environ Res Public Health. 2021 Jul 25;18(15):7869.
4. WHO. 2011. Global recommendations on diet, physical activity and health. Physical activity for adults. Avaliable from: http://www.who.int/dietphysicalactivity/factsheet adults/en/
5. Grujičić M, Ilić M, Novaković B, Vrkatić A, Lozanov-Crvenković Z. Prevalence and Associated Factors of Physical Activity among Medical Students from the Western Balkans. Int J Environ Res Public Health. 2022 Jun 23;19(13):7691.
6. Warren JM, Ekelund U, Besson H, Mezzani A, Geladas N, Vanhees L. Assessment of physical activity – a review of methodologies with reference to epidemiological research: a report of the exercise physiology section of the European Association of Cardiovascular Prevention and Rehabilitation. Eur J Cardiovasc Prev Rehabil. 2010;17(2):127–39.
7. Dąbrowska-Galas M, Ptaszkowski K, Dąbrowska J. Physical Activity Level, Insomnia and Related Impact in Medical Students in Poland. Int J Environ Res Public Health. 2021 Mar 17;18(6):3081.
8. Gallo LA, Gallo TF, Young SL, Fotheringham AK, Barclay JL, Walker JL, et al. Adherence to Dietary and Physical Activity Guidelines in Australian Undergraduate Biomedical Students and Associations with Body Composition and Metabolic Health: A Cross-Sectional Study. Nutrients. 2021 Oct 3;13(10):3500.
9. Mašina T, Madžar T, Musil V, Milošević M. Differences in Health-Promoting Lifestyle Profile Among Croatian Medical Students According to Gender and Year of Study. Acta Clin Croat. 2017 Mar;56(1):84-91.
10. Alhammad SA, Almutairi FM, Bajsair AS, Alghamdi AS, Algarni FS, Aldaihan MM, et al.Physical activity levels among undergraduate students at the College of Applied Medical Sciences, King Saud University, Riyadh: A prevalence study. Medicine (Baltimore). 2023 Dec 1;102(48):e36386.
11. Abrantes LCS, de Souza de Morais N, Gonçalves VSS, Ribeiro SAV, de Oliveira Sediyama CMN, do Carmo Castro Franceschini S, et al. Physical activity and quality of life among college students without comorbidities for cardiometabolic diseases: systematic review and meta- analysis. Qual Life Res. 2022 Jul;31(7):1933-1962.
12. Ilić M, Pang H, Vlaški T, Grujičić M, Novaković B. Motives and Barriers for Regular Physical Activity among Medical Students from the Western Balkans (South-East Europe Region). Int J Environ Res Public Health. 2022 Dec 4;19(23):16240.
13. Yadav M. Diet, Sleep and Exercise: The Keystones of Healthy Lifestyle for Medical Students. JNMA J Nepal Med Assoc. 2022 Sep 1;60(253):841-843.
14. Troyer D, Ullrich IH, Yeater RA, Hopewell R. Physical activity and condition, dietary habits, and serum lipids in second-year medical students. J Am Coll Nutr. 1990 Aug;9(4):303- 7.
15. Webb E, Ashton CH, Kelly P, Kamah F. An update on British medical students' lifestyles. Med Educ. 1998 May;32(3):325-31.
16. Bergeron N, Al-Saiegh S, Ip EJ. An Analysis of California Pharmacy and Medical Students' Dietary and Lifestyle Practices. Am J Pharm Educ. 2017 Oct;81(8):5956.
17. Memon AR, Gupta CC, Crowther ME, Ferguson SA, Tuckwell GA, Vincent GE. Sleep and physical activity in university students: A systematic review and meta-analysis. Sleep Med Rev. 2021 Aug; 58:101482.
18. Ainsworth, B. E., et al. (2000). "Comparison of three methods for measuring the time spent in physical activity." Med Sci Sports Exerc 32: S457-64.
19. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. J Gen Intern Med. 2001 Sep;16(9):606-13.
20. Zung WW. A rating instrument for anxiety disorders. Psychosomatics. 1971 Nov-Dec;12(6):371-9.
21. Pop LM, Iorga M, Şipoş LR, Iurcov R. Gender Differences in Healthy Lifestyle, Body Consciousness, and the Use of Social Networks among Medical Students. Medicina (Kaunas). 2021 Jun 24;57(7):648.
22. Martinovic D, Tokic D, Vilovic M, Rusic D, Bukic J, Bozic J. Sport Dietary Supplements and Physical Activity in Biomedical Students. Int J Environ Res Public Health. 2021 Feb 19;18(4):2046.
23. Sakamaki R, Toyama K, Amamoto R, Liu CJ, Shinfuku N. Nutritional knowledge, food habits and health attitude of Chinese university students--a cross sectional study. Nutr J. 2005 Feb 9; 4:4.
24. Musaiger AO, Awadhalla MS, Al-Mannai M, AlSawad M, Asokan GV. Dietary habits and sedentary behaviors among health science university students in Bahrain. Int J Adolesc Med Health. 2017 Apr 1;29(2).
25. McFadden T, Fortier M, McGinn R, Levac BM, Sweet SN, Tomasone JR. Demographic differences in Canadian medical students' motivation and confidence to promote physical activity. Fam Pract. 2020 Feb 19;37(1):56-62.
26. Oyeyemi AL, Muhammed S, Oyeyemi AY, Adegoke BOA. Patterns of objectively assessed physical activity and sedentary time: Are Nigerian health professional students complying with public health guidelines? PLoS One. 2017 Dec 27;12(12):e0190124.
27. Anand T, Tanwar S, Kumar R, Meena GS, Ingle GK. Knowledge, attitude, and level of physical activity among medical undergraduate students in Delhi. Indian J Med Sci. 2011 Apr;65(4):133-42.
28. Romero-Blanco C, Rodríguez-Almagro J, Onieva-Zafra MD, Parra-Fernández ML, Prado- Laguna MDC, Hernández-Martínez A. Physical Activity and Sedentary Lifestyle in University Students: Changes during Confinement Due to the COVID-19 Pandemic. Int J Environ Res Public Health. 2020 Sep 9;17(18):6567.
29. Molanorouzi, K., Khoo, S. & Morris, T. Motives for adult participation in physical activity: type of activity, age, and gender. *BMC Public Health* 15, 66 (2015).
30. Pope HG Jr, Gruber AJ, Mangweth B, Bureau B, deCol C, Jouvent R, Hudson JI. Body image perception among men in three countries. Am J Psychiatry. 2000 Aug;157(8):1297-301.
31. Brown, C.E.B., Richardson, K., Halil-Pizzirani, B. *et al.* Key influences on university students' physical activity: a systematic review using the Theoretical Domains Framework and the COM-B model of human behaviour. *BMC Public Health* 24, 418 (2024).
32. Zeńczak-Praga K, Pluto-Prondzinska J, Zgorzalewicz-Stachowiak M. Medicine and Physiotherapy students: are they physically active? Comparative research on Spanish and German population. Pol Merkur Lekarski. 2017 May 23;42(251):205-209.
33. Bergier J, Tsos A, Popovych D, Bergier B, Niźnikowska E, Ács P, et al. Level of and Factors Determining Physical Activity in Students in Ukraine and the Visegrad Countries. Int J Environ Res Public Health. 2018 Aug 13;15(8):1738.
34. Trivedi T, Liu J, Probst J, Merchant A, Jhones S, Martin AB. Obesity and obesity-related behaviors among rural and urban adults in the USA. Rural Remote Health. 2015 Oct- Dec;15(4):3267.
35. Siegmann P, Teismann T, Fritsch N, Forkmann T, Glaesmer H, Zhang XC, et al. Resilience to suicide ideation: A cross-cultural test of the buffering hypothesis. Clin Psychol Psychother. 2018 Jan;25(1):e1-e9.
36. Bize R, Johnson JA, Plotnikoff RC. Physical activity level and health-related quality of life in the general adult population: a systematic review. Prev Med. 2007 Dec;45(6):401-15.
37. Callaghan P. Exercise: a neglected intervention in mental health care? J Psychiatr Ment Health Nurs. 2004 Aug;11(4):476-83.
38. Lebensohn P, Dodds S, Benn R, Brooks AJ, Birch M, Cook P, et al. Resident wellness behaviors: relationship to stress, depression, and burnout. Fam Med. 2013 Sep;45(8):541-9.
39. Sloan RA, Sawada SS, Martin CK, Church T, Blair SN. Associations between cardiorespiratory fitness and health-related quality of life. Health Qual Life Outcomes. 2009 May 28; 7:47.
40. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. Compr Physiol. 2012 Apr;2(2):1143-211.

MENTALNO BLAGOSTANJE STUDENATA MEDICINE: ČINE LI STILOVI ŽIVOTA I FIZIČKA AKTIVNOST RAZLIKU?

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Sažetak

Uvod: Životni stil, koji obuhvata navike u ishrani, fizičku aktivnost, spavanje, pušenje i konzumiranje alkohola između ostalih faktora utiče na zdravlje pojedinca, ali i populacije u celini. Studenti medicine često imaju suboptimalan nivo fizičke aktivnosti i nezdrave životne navike, što može uticati na njihovo blagostanje i buduću medicinsku praksu. Cilj ovog istraživanja bilo je ispitivanje učestalosti nedovoljne fizičke aktivnosti i faktora stila života povezanih sa njom među studentima pete godine Medicinskog fakulteta Univerziteta u Beogradu, kao i njihovog međusobnog uticaja na fizičko i mentalno zdravlje studenata. **Metode:** Studija preseka je sprovedena među studentima pete godine Medicinskog fakulteta Univerziteta u Beogradu tokom jedne nedelje na vežbama iz Socijalne medicine u novembru 2023. godine. Instrument istraživanja bio je upitnik koji su studenti anonimno popunili. Studija je uključila 432 učesnika, sa obuhvatom od 90,4%. Ispitivani su: fizička aktivnost, karakteristike stila života, simptomi depresivnosti i anksioznosti. Na osnovu energetske potrošnje studenti su bili podeljeni u kategorije sa dovoljnom i nedovoljnom fizičkom aktivnošću.

Rezultati: Ukupno 379 studenata (92%) bilo je u grupi sa dovoljnom fizičkom aktivnošću, dok je njih 33 (8%) bilo u grupi sa nedovoljnom fizičkom aktivnošću. Multivarijantnom logističkom regresijom pokazana je značajna povezanost fizičke neaktivnosti sa manjim BMI (OR: 0,81, 95% CI: 0,69-0,95) i izraženijim simptomima depresije (OR: 1,07, 95% CI: 1,01-1,15) i lošijim finansijskim statusom (OR: 0,51, 95% CI: 0,27-0,96).

Zaključak: Većina ispitivanih studenata je dovoljno fizički aktivna. Fizička neaktivnost bila je značajno povezana sa manjim BMI, izraženijim simptomima depresije i lošijim finansijskim statusom.

Ključne reči: fizička aktivnost, stilovi života, gojaznost, mentalno zdravlje, studenti medicine

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ORIGINAL ARTICLE

A novel support vector machine learning approach using fractal and run-length matrix indicators for identifying nuclear changes in laryngeal cancer

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Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction/Aim: We aimed to propose a novel and innovative concept of a support vector machine learning algorithm that employs fractal and run-length matrix indicators of nuclear structure to identify malignant squamous epithelial cells in laryngeal cancer.

Material and Methods: Regions of interest in micrographs of laryngeal cancer and chronic laryngitis were analyzed using the box-counting fractal and run-length matrix textural techniques. For each nucleus, we quantified fractal dimension values, lacunarity, long-run emphasis, and short-run emphasis. These features were used as input data for training and testing the support vector machine model in the "Scikit-learn" library for Python.

Results: The support vector machine model produced relatively good performance indicators. The classification accuracy of the model was 0.83, indicating its adequate ability to distinguish cancer cells from non-cancer cells in our sample. The F1 score (the harmonic mean of precision and recall) was 0.83, suggesting a relatively good balance between these two metrics. The value of the Matthews Correlation Coefficient for this model was 0.65, which indicated moderate agreement between the predicted and actual labels and balanced performance across the two classes.

Conclusion: The proposed model provides a solid foundation for further developing artificial intelligence systems for signal analysis in cancer research. If the limitations of this concept are addressed, future research can focus on developing a more comprehensive machine-learning model for identifying laryngeal epithelial cancer cells.

Keywords: artificial intelligence, machine learning, nucleus, chromatin, fractal

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INTRODUCTION

Novel Information and Communication Technologies (ICTs) have enabled a significant increase in the level of automation when analyzing physiological and pathological signals. One such technology is support vector machine (SVM) learning for identification of patterns present in two-dimensional signal data (1, 2). SVM is a supervised learning approach where a computer model during development is presented with numerous correctly associated inputs and outputs. In time, the model learns of new associations and patterns and can predict the correct outcome based on the new inputs. In the case of SVM, the model uses a decision boundary, a line, a plane, or a hyperplane to divide data points, which is particularly useful for classification tasks. In biomedical sciences, the input data usually belong to multidimensional spaces so hyperplane is commonly used for the separation of data points. The closest data points with the greatest impact on the shape and location of the hyperplane are often referred to as support vectors (3, 4).

The SVM approach is commonly used in clinical medicine to discriminate between physiological and pathological states and conditions. Here, various kernel functions can be used to separate data, including polynomial, sigmoid, and radial basis kernel functions. Inputs can include data on two-dimensional patterns such as cell size and shape, fractal parameters, textural indicators and wavelet transform quantifiers. The outputs can include a class of the cell (i.e., damaged or intact), patient status, diagnostic category, prognostic indicators, and other data that can represent a descriptor of a data class. Less frequently, SVMs can be built for regression purposes, for prediction of a continuous physiological or pathological variable (5, 6).

When using SVM and machine learning in general for identification of data patterns associated with cancer, probably the best approach would be to apply a mathematical analysis of textural or related changes in cell structure. Previously, such concepts have been introduced in machine learning, particularly in applying co-occurrence matrix analysis for the training of supervised machine learning models and the models based on decision trees (7). Apart from the co-occurrence matrix, run-length matrix (RLM) and fractal approaches also have certain potential in training both SVM and alternative models for cell classification in other pathologies (8, 9). The potential rationale for using SVM would be its greater ability to handle high-dimensional spaces and better utilization of computational and processing power.

In our previous work, we have demonstrated that, based on wavelet and gray-level co-occurrence matrix (GLCM) textural data related to nuclear organization, it is possible to train SVM and random forest models that can be useful for the identification of squamous epithelial cancer cells in laryngeal cancer (LC) tissue (7). This research further raised questions if such a model can be developed to use other types of inputs. Hereby, we present a concept of an SVM model that utilizes a combination of nuclear fractal and run-length matrix parameters in order to differentiate between intact and malignant squamous epithelial cells (SECs) in laryngeal tissue. The proposed model uses 4 mathematical parameters: fractal dimension, lacunarity, RLM short-run emphasis, and RLM long-run emphasis. Our initial results indicate that when applied to nuclear regions of interest, the developed SVM model may have satisfactory performance.

MATERIALS AND METHODS

The research is a continuation of our previous work (7), where digital micrographs were obtained from biopsy samples of 50 patients diagnosed with laryngeal squamous cell carcinoma and the control group consisting of 50 patients established to have only chronic laryngitis. The patients were previously diagnosed and treated at the University Clinical Hospital Center Zemun, Belgrade, Serbia, and the researchers obtained approval from the Ethical Commission of the University of Belgrade, Faculty of Medicine Serbia (Approval No. 17/I-17, 12-Jan-2023.). Inclusion and exclusion criteria for this retrospective study as well as micrograph creation and characteristics were explained earlier (7).

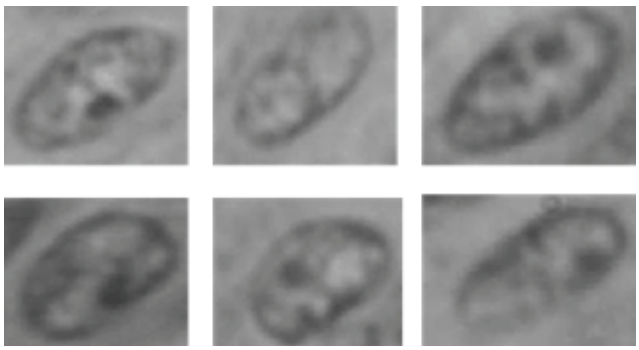

Figure 1. Example of squamous epithelial cell (SEC) nuclei in a format suitable for binarization and fractal analysis using the box-counting method.

Fractal and run-length matrix analysis was performed on a total of 2000 SEC nuclear regions of interest (ROIs), of which 1000 belonged to the experimental and 1000 to the control group. The contrast, brightness and other image indicators were previously adjusted to achieve the optimal binarization thresholds and performance of fractal analysis (Figure 1). For the fractal analysis of cell nuclei, we used FracLac software, previously developed by Audrey Karperien and Charles Sturt University (10, 11). Fractal dimension (D) was calculated using the traditional box-counting technique (10, 12-14) where the object is covered by a multitude of boxes of different sizes (ε) after which the calculations are done from a slop of

logarithmic regression line where box numbers (N) and the sizes are taken into account:

$$D=\lim\varepsilon \rightarrow 0[\log N_{\varepsilon}/\log \varepsilon$$

While fractal dimension is an indicator of complexity, fractal lacunarity (λ) is an indicator of heterogeneity and largely depends on the number, size and other characteristics of architectural gaps. It is determined from the variation coefficient (CV) for resolution unit mass considering the grid position (g) and scale:

$$\lambda = CV_{\varepsilon,g}{}^{2} = (\sigma_{\varepsilon,g}/_{\mu\varepsilon,g})^{2}$$

Run-length matrix analysis was done in MaZda software (version 4.6) created by the authors at the Institute of Electronics, Technical University of Lodz (TUL), Poland (15-18). Briefly, this method analyzes sequences of consecutive resolution units (also called "runs") in order to quantify elements of textural anisotropy, as well as other aspects of spatial relationships within the two-dimensional signals. In this work, we focused on two major quantifiers of RLM, Long Run Emphasis (LngREmph) and Short Run Emphasis (ShrtREmph). Both indicators are dependent of respective frequencies of runs with length j that have a gray value of i, or p(i,j):

$$\text{ShrtREmph} = \left(\sum_{i=1}^{N_g}\sum_{j=1}^{N_r}\frac{p(i,j)}{j^2}\right)/C$$

$$\text{LngREmph} = \left(\sum_{i=1}^{N_g}\sum_{j=}^{N_r} j^2 p(i,j)\right)/C$$

In these formulas, coefficient C is determined as:

$$C = \sum_{i=1}^{N_g}\sum_{j=1}^{N_r} p(i,j)$$

Support vector machine algorithm was proposed within the Google Colab and Jupyter Notebook service, using the scikit-learn machine learning library (version 1.3) for Python programming language (19-22). The obtained values of nuclear ROI fractal dimension, lacunarity, LngREmph and ShrtREmph were used as input parameters, while the output consisted of the cell class related to its affiliation to the experimental or the control group. P-dimensional mathematical vectors were the representations of specific data points. Consequently, separation of the data was achieved using a (p-1)-dimensional hyperplane(s). For the model, in Scikit-learn library, we calculated the values of the F1 score (harmonic mean of precision and recall), Matthews Correlation Coefficient (MCC, measure of the quality of binary classifications), and the area under the Receiver Operating Characteristic (ROC) curve, a performance measurement for classification problems at various thresholds settings. Matplotlib, a plotting library for Python was used for visualizing model performance. NumPy (Numerical Python), a core scientific computing library, was used for numerical computation and other data operations.

RESULTS

All four parameters that we quantified in this study were suitable for supervised machine learning purposes and for the development of support vector machine models. The average nuclear ROI long run emphasis value was 5.417 ± 1.997 in the LC group and 16.134 ± 10.479 in the controls. Conversely, values of short-run emphasis were lower in controls compared to the LC group and equaled 0.461 ± 0.085 and 0.505 ± 0.093, respectively. A statistically highly significant difference ($p<0.01$) was observed between the groups for both run-length matrix indicators. We also observed significant differences between the groups in values of fractal parameters of cell nuclei ($p<0.01$). The average nuclear ROI fractal dimension value was 1.522 ± 0.130 in the LC group and 1.585 ± 0.119 in the controls. The average value of fractal lacunarity was 0.505 ± 0.093 in the LC group and 0.461 ± in the controls. These results indicated that the nuclear patterns in squamous epithelial cells in laryngeal cancer tissue are characterized by reduced complexity and possibly increased levels of fractal heterogeneity.

After 5-fold cross-validation, the support vector machine model produced relatively good performance indicators. The classification accuracy of the model equaled 0.83, suggesting the adequate ability of the model to separate cancer from non-cancer cells in our sample. The precision and recall of the model were 0.79 and 0.87, respectively, highlighting the model's strong performance in the identification of true positives but with room for improvement. The value of the F1 score (harmonic mean of precision and recall) was 0.83, indicating that there is a relatively good balance between the two metrics. The value of the Matthews Correlation Coefficient for this model was 0.65, which suggested a moderate agreement between the predicted and actual labels and balanced performance across the two classes. The area under the Receiver Operating Characteristics curve was 0.89 which suggested a good discriminatory power of the SVM classifier. The ROC curve is presented in **Figure 2**.

Feature importance analysis was performed taking into account the best hyperparameters during hyperparameter optimization. For linear kernel, the most influential feature for model development and performance was the fractal dimension, with a score of 1.78. The second most important feature was nuclear ROI lacunarity, with a score of 1.50. Short-run emphasis had a fracture importance of 1.44, while long-run emphasis had a feature importance of 0.31. During the creation of the support vector machine learning model, we also performed the stratified k-fold cross-validation on five splits with the values of the

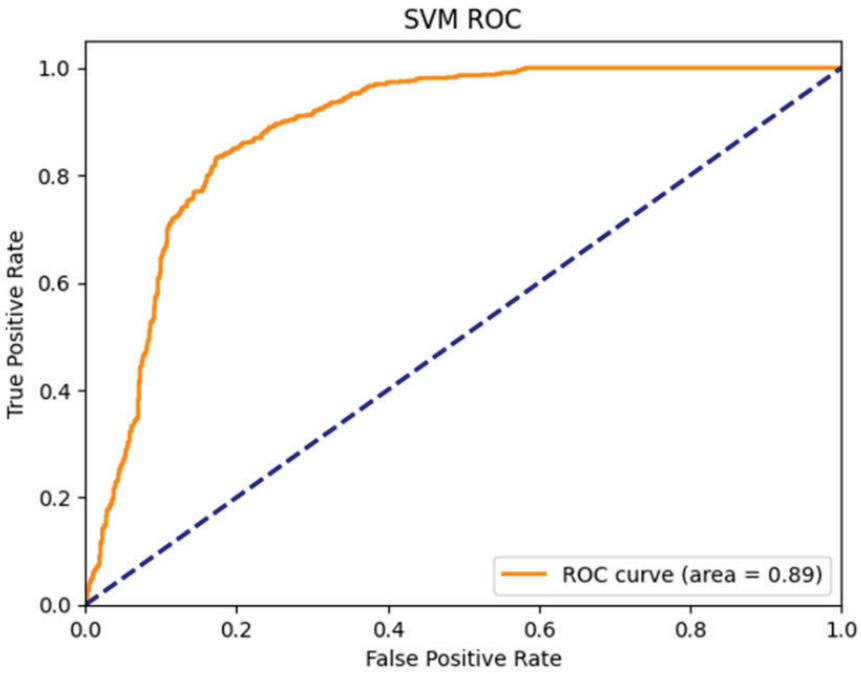

Figure 2. Receiver Operating Characteristics curve of the support vector machine model.

"shuffle" parameter set to "True" in order to increase the degree of randomness. Various hyperparameters for this model have been considered, including the Regularization parameter, shrinking heuristic, Tolerance for stopping criteria, Maximum number of iterations, and Kernel types. The proposed segment of the code is shown in **figure 3**.

DISCUSSION

In this work, we explain the concept of a support vector machine algorithm intended for differentiation between malignant and non-malignant laryngeal squamous epithelial cells. We present a model that functions based on fractal and run-length matrix inputs obtained through mathematical analysis of cell nuclear architecture. The quantified indicators of the model performance suggest that the SVM concept, as a whole, in these clinical and experimental settings, holds some potential, especially when considering its future integration with similar artificial intelligence-based computational systems.

This research is a continuation of our recently published work on the application of nuclear GLCM and wavelet mathematical inputs for supervised machine learning (7). There, we created an SVM model using nuclear inverse difference moment, angular second moment, contrast, correlation, sum variance, as well as coefficient energies of the discrete wavelet transform. Similarly, in our present study, the SVM approach was used to separate malignant from non-malignant laryngeal squamous epithelial cells. The developed SVM model demonstrated very good performance since its classification accuracy was 83% and the area under the receiver operating characteristic curve was 0.89. This performance was similar to the alternative decision tree–based, random forest model described in the previous research (7).

```
from sklearn.svm import SVC
from sklearn.model_selection import cross_val_predict, cross_val_score, StratifiedKFold, GridSearchCV
from sklearn.metrics import roc_curve, auc, f1_score, precision_score, recall_score, matthews_corrcoef, classification_report, accuracy_score
import matplotlib.pyplot as plt
import numpy as np

X = df[['Horzl_LngREmph', 'Horzl_ShrtREmp', 'FD', 'Lac']]
y = df['target']


param_grid = {
    'C': [0.01, 0.1, 1, 10, 100, 1000],
    'kernel': ['linear', 'rbf', 'poly', 'sigmoid'],
    'gamma': ['scale', 'auto', 0.001, 0.01, 0.1, 1],
    'degree': [2, 3, 4, 5],
    'coef0': [0, 0.1, 0.5, 1, 2],
    'shrinking': [True, False],
    'class_weight': [None, 'balanced'],
    'tol': [1e-4, 1e-3, 1e-2],
    'max_iter': [-1, 1000, 5000]
}
```

Figure 3. The segment of the Python Scikit-learn code with optional hyperparameter tuning.

This is not the first research where the run-length matrix and fractal parameters of cell nuclei have been used for machine learning classification tasks. In 2023, the values of fractal dimension were considered as inputs for the creation of an SVM classifier for the detection of structural changes of cell nuclei following exposure to a hyperosmotic environment (23). When combining this indicator with GLCM and DWT quantifiers, the resulting model was shown to have a relatively good classification accuracy of 71.7%. Although this performance is not considered excellent, it may still hold scientific potential since human-based identification of structural changes is much less effective. In recent work, nuclear textural features based on run length matrix and wavelet analyses were used for the detection of discrete changes in chromatin distribution associated with iron nanoparticle exposure (8). Therein, it was shown that for hepatocytes, it is possible to create a supervised machine learning model, specifically those based on random forest and gradient boosting architecture, to distinguish between intact and possibly damaged cells.

The rationale for using run-length matrix and fractal parameters for the detection of change in nuclear structure lies in the fact that these techniques can objectively quantify structural alterations in digital micrographs and their regions of interest. This is particularly the case with ROIs of nuclei and nuclear chromatin, where the changes are almost invisible to the human eye during a conventional microscopy assessment. Nuclear chromatin distribution is governed by numerous intracellular and intranuclear mechanisms (24-27), and cells exposed to toxic environments or malignant transformation may exhibit changes in euchromatin and heterochromatin patterns detectable using contemporary computational methods. In the case of cancer cells, one can expect significant alterations in nuclear morphology, which are often noticeable by the human eye and may include extensive redistribution of heterochromatin, chromosomal rearrangements, and translocations, as well as chromatin marginalization. Also, in malignant cells, nuclei may have irregular contours or be distorted, and overall DNA content and density may be increased, leading to a phenomenon called hyperchromasia. Finally, nucleoli may be prominent, enlarged and more conspicuous. These alterations may be detectable using fractal and textural methods, particularly bearing in mind that in the past, the techniques have been successfully used for the detection of nuclear changes in both physiological and pathological conditions (28-30).

Limitations of our approach include the relatively small ROI sample size used for training the model, as well as the usual drawbacks of the development of machine learning models in biomedical research. Support vector machines, although relatively useful when handling multidimensional data in medicine, may still suffer from generalizability to other cell populations and clinical conditions. In other words, the model may adequately identify and classify cancer cells in this sample of malignant squamous epithelial cells, but this effectiveness may not be manifested in other circumstances. Also, like in many other approaches in supervised machine learning, the interpretability of SVMs remains relatively low since the inner workings of the model, due to its multidimensionality and overall complexity, remain elusive. Finally, the fact that the model is focused on cell nuclear ROIs rather than on the patient and technical issues related to the reproducibility of fractal and RLM analysis also pose a significant limitation. Therefore, our results and the SVM approach should be considered more as a preliminary concept than a fully applicable and developed machine learning model.

CONCLUSION

In conclusion, we present a support vector machine learning concept intended to differentiate between nuclear regions of interest of malignant and non-malignant laryngeal squamous epithelial cells. The model is based on nuclear values of fractal dimension, fractal lacunarity, run-length matrix short-run emphasis, and long-run emphasis. Preliminary results show that the model could reach acceptable discriminatory power and other measures of performance, giving it the potential to be integrated with other machine-learning approaches for the identification of cancer cells. If the limitations of this concept are overcome, future research can be focused on the development of a more comprehensive and effective artificial intelligence system based on run-length matrix and fractal indicators with potential applications in clinical medicine.

Conflicts of interest

None to declare.

Author contributions

SV and IP contributed to the conception and design of the work, the acquisition, analysis, and interpretation of data, and the preparation of the manuscript draft. MBJ and JPP contributed to the conception and design of the work and the preparation of the manuscript draft.

Ethical approval

The researchers obtained approval from the Ethical Commission of the University of Belgrade, Faculty of Medicine Serbia (Approval No. 17/I-17, 12-Jan-2023.). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

REFERENCES

1. Xu L, Liang G, Shi S, Liao C. SeqSVM: A Sequence-Based Support Vector Machine Method for Identifying Antioxidant Proteins. Int J Mol Sci. 2018;19(6).
2. Cai CZ, Han LY, Ji ZL, Chen X, Chen YZ. SVM-Prot: Web-based support vector machine software for functional classification of a protein from its primary sequence. Nucleic Acids Res. 2003;31(13):3692-7.
3. Lee HK, Kim CH, Bhattacharjee S, Park HG, Prakash D, Choi HK. A Paradigm Shift in Nuclear Chromatin Interpretation: From Qualitative Intuitive Recognition to Quantitative Texture Analysis of Breast Cancer Cell Nuclei. Cytometry A. 2020.
4. AlKubeyyer A, Ben Ismail MM, Bchir O, Alkubeyyer M. Automatic detection of the meningioma tumor firmness in MRI images. J Xray Sci Technol. 2020;28(4):659-82.
5. Jiang H, Ching WK. Classifying DNA repair genes by kernel-based support vector machines. Bioinformation. 2011;7(5):257-63.
6. Vidya KS, Ng EY, Acharya UR, Chou SM, Tan RS, Ghista DN. Computer-aided diagnosis of Myocardial Infarction using ultrasound images with DWT, GLCM and HOS methods: A comparative study. Comput Biol Med. 2015;62:86-93.
7. Valjarevic S, Jovanovic MB, Miladinovic N, Cumic J, Dugalic S, Corridon PR, et al. Gray-Level Co-occurrence Matrix Analysis of Nuclear Textural Patterns in Laryngeal Squamous Cell Carcinoma: Focus on Artificial Intelligence Methods. Microscopy and Microanalysis. 2023;29(3):1220-7.
8. Paunovic Pantic J, Vucevic D, Radosavljevic T, Corridon PR, Valjarevic S, Cumic J, et al. Machine learning approaches to detect hepatocyte chromatin alterations from iron oxide nanoparticle exposure. Scientific reports. 2024;14(1):19595.
9. Pantic I, Paunovic Pantic J. Artificial Intelligence in Chromatin Analysis: A Random Forest Model Enhanced by Fractal and Wavelet Features. Fractal and Fractional. 2024;8(8):490.
10. Karperien AL, Jelinek HF. Box-Counting Fractal Analysis: A Primer for the Clinician. Adv Neurobiol. 2024;36:15-55.
11. Karperien A. FracLac for ImageJ. 1999-2023 [Available from: http://rsb.info.nih.gov/ij/plugins/fraclac/FLHelp/Introduction.htm.
12. Revittser A, Selin I, Negulyaev Y, Chubinskiy-Nadezhdin V. The analysis of F-actin structure of mesenchymal stem cells by quantification of fractal dimension. PloS one. 2021;16(11):e0260727.
13. Eser S, Saribas E. Anatomical assessment of the trabecular structure of the alveolar bone in periodontal disease by fractal analysis method. Folia Morphol (Warsz). 2024;83(1):157-67.
14. Yavuz E, Yardimci S. Comparison of periapical radiography, panoramic radiography, and CBCT in the evaluation of trabecular bone structure using fractal analysis. Oral Radiol. 2024.
15. Strzelecki M, Szczypinski P, Materka A, Klepaczko A. A software tool for automatic classification and segmentation of 2D/3D medical images. Nuclear Instruments & Methods In Physics Research A. 2013;702:137-40.
16. Szczypinski P, Strzelecki M, Materka A, Klepaczko A. MaZda-A software package for image texture analysis. Computer methods and programs in biomedicine. 2009;94(1):66-76.
17. Szczypinski P, Strzelecki M, Materka A. MaZda - a Software for Texture Analysis. Proc of ISITC 2007, November 23-23, 2007; Republic of Korea2007. p. 245-9.
18. Kociołek M, Materka A, Strzelecki M, Szczypinski P, editors. Discrete wavelet transform –derived features for digital image texture analysis. Proc of Interational Conference onSignals and Electronic Systems, 18-21 September 2001; 2001; Lodz, Poland.
19. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: Machine Learning in Python. J Mach Learn Res. 2011;12:2825–30.
20. Ahn S. Building and analyzing machine learning-based warfarin dose prediction models using scikit-learn. Transl Clin Pharmacol. 2022;30(4):172-81.
21. Kondo Y. [[Fundamentals] 6. Calculation of Machine Learning-related Statistics and Evaluation Values Using Scikit-learn]. Nihon Hoshasen Gijutsu Gakkai Zasshi. 2023;79(12):1387-94.
22. Yang Y, Hu P, Chen SR, Wu WW, Chen P, Wang SW, et al. Predicting the Activity of Oral Lichen Planus with Glycolysis-related Molecules: A Scikit-learn-based Function. Curr Med Sci. 2023;43(3):602-8.
23. Pantic I, Valjarevic S, Cumic J, Paunkovic I, Terzic T, Corridon PR. Gray Level Co-Occurrence Matrix, Fractal and Wavelet Analyses of Discrete Changes in Cell Nuclear Structure following Osmotic Stress: Focus on Machine Learning Methods. Fractal and Fractional. 2023;7(3):272.
24. Pantic I, Paunovic Pantic J. Artificial Intelligence in Chromatin Analysis: A Random Forest Model Enhanced by Fractal and Wavelet Features. Fractal and Fractional [Internet]. 2024; 8(8).
25. Espinoza JA, Kanellis DC, Saproo S, Leal K, Martinez JF, Bartek J, et al. Chromatin damage generated by DNA intercalators leads to degradation of RNA Polymerase II. Nucleic Acids Res. 2024.
26. Minami K, Iida S, Maeshima K. Chromatin organization and DNA damage. Enzymes. 2022;51:29-51.
27. Wang D, Lu X, Jiang Y, Pan L, Zhu F, Yu A, et al. The chromatin remodeling protein BPTF mediates cell cycle, proliferation and apoptosis in porcine ovarian granulosa cells. Theriogenology. 2023;211:172-81.
28. Mattos AC, Florindo JB, Adam RL, Lorand-Metze I, Metze K. The Fractal Dimension Suggests Two Chromatin Configurations in Small Cell Neuroendocrine Lung Cancer and Is an Independent Unfavorable Prognostic Factor for Overall Survival. Microsc Microanal. 2022:1-5.
29. Dincic M, Todorovic J, Nesovic Ostojic J, Kovacevic S, Dunderovic D, Lopicic S, et al. The Fractal and GLCM Textural Parameters of Chromatin May Be Potential Biomarkers of Papillary Thyroid Carcinoma in Hashimoto's Thyroiditis Specimens. Microsc Microanal. 2020;26(4):717-30.
30. Gupta S, Savala R, Gupta N, Dey P. Fractal dimension and chromatin textural analysis to differentiate follicular carcinoma and adenoma on fine needle aspiration cytology. Cytopathology. 2020;31(5):491-3.

NOV PRISTUP MAŠINSKOM UČENJU POMOĆU POTPORNIH VEKTORA KOJI KORISTI FRAKTALNE I MATRIČNE POKAZATELJE DUŽINE NIZA ZA IDENTIFIKACIJU JEDARNIH PROMENA KOD KARCINOMA LARINKSA

Svetlana Valjarević[1], Milan B. Jovanović[1], Jovana Paunović Pantić[2], Igor Pantić[3, 4]

Sažetak

Uvod/Cilj: Cilj istraživanja je bio da predložimo novi i inovativni koncept algoritma mašinskog učenja sa potpornim vektorima koji koristi fraktalne i matrične pokazatelje dužine niza strukture jedra za identifikaciju malignih skvamoznih epitelnih ćelija kod laringealnog karcinoma.

Materijal i metode: Analizirane su regije od interesa na mikrografima laringealnog karcinoma i hroničnog laringitisa korišćenjem fraktalne tehnike brojanja kvadrata i teksturalne tehnike matrice dužine niza. Za svako jedro kvantifikovane su vrednosti fraktalne dimenzije, lakunarnosti, naglašenosti dugih nizova i naglašenosti kratkih nizova. Ove karakteristike korišćene su kao ulazni podaci za treniranje i testiranje modela mašinskog učenja sa potpornim vektorima u biblioteci *Scikit-learn* za Python.

Rezultati: Model mašinskog učenja sa potpornim vektorima pokazao je relativno dobre pokazatelje performansi. Tačnost klasifikacije modela iznosila je 0,83 što ukazuje na adekvatnu sposobnost modela da razlikuje kancerogene od nekancerogenih ćelija u našem uzorku. Vrednost F1 ocene (harmonijska sredina preciznosti i osetljivosti) iznosila je 0,83, što sugeriše relativno dobar balans između ova dva pokazatelja. Vrednost Metjuzovog koeficijenta korelacije za ovaj model iznosila je 0,65 što ukazuje na umerenu saglasnost između predviđenih i stvarnih oznaka i uravnotežene performanse modela unutar dve klase.

Zaključak: Predloženi model pruža solidnu osnovu za dalji razvoj sistema veštačke inteligencije za analizu signala u istraživanjima raka. Ako se ograničenja ovog koncepta prevaziđu, buduća istraživanja mogu biti usmerena na razvoj sveobuhvatnijeg modela mašinskog učenja za identifikaciju epitelnih ćelija laringealnog karcinoma.

Ključne reči: veštačka inteligencija, mašinsko učenje, jedro, hromatin, fraktal

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ORIGINAL ARTICLE

The influence of cancer stage and surgery extent on long-term outcomes in ovarian cancer patients

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Summary

Introduction: Standard therapy for patients with ovarian cancer involves cytoreductive surgery followed by platinum-based chemotherapy. Factors such as the stage of the disease at diagnosis, the histological type of the tumor, the size of the tumor and the presence of residual disease after cytoreductive surgery influence the prognosis of patients.

Material and methods: This scientific paper presents a retrospective study in which the following parameters were analyzed by analyzing data obtained from the documentation of patients treated for ovarian cancer at the Institute of Oncology and Radiology of Serbia and the Institute of Oncology of Vojvodina: age of patients, period from last chemotherapy to disease progression, number of bevacizumab cycles received, disease stage, reasons for discontinuation of bevacizumab, type of surgery, and pathological verification of the disease.

Results: We have shown that PFS is longer in the operated group (suboptimal operation) compared to those who were not operated ($p < 0.05$, Log-Rank test). There is no statistically significant difference in PFS between stages IIIc and IV, as determined by Log-Rank test. Additionally, in our research, there was no significant difference in the number of operated patients based on disease stage IIIc or IV ($p = 0.361$).

Conclusions: Our data show that cytoreduction appears to confer a survival advantage in women with ovarian cancer treated with a combination of bevacizumab and chemotherapy. New studies should show whether the stage of the disease plays a significant role in the survival of patients with ovarian cancer.

Key words: ovarian cancer, progression-free survival, type of surgery, disease stage

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INTRODUCTION

Ovarian cancer is among the most common gynecologic malignancies, with over 300,000 cases diagnosed worldwide in 2020. Unfortunately, it is often detected at advanced stages due to non-specific symptoms and the absence of effective screening methods. Ovarian cancer is a very aggressive disease that, despite new therapies, does not increase the number of cured women. The standard treatment for ovarian cancer includes cytoreductive surgery followed by adjuvant platinum-based chemotherapy. Prognosis is influenced by clinical and biological factors such as tumor stage and grade at diagnosis, tumor size, and the presence of residual disease after cytoreductive surgery (1, 2).

Overall survival (OS) is considered the gold standard endpoint in cancer clinical trials because it is an objective measure, it can be accurately documented through the date of death, and is less prone to reporting bias. However, achieving statistically significant OS results typically requires large-scale clinical trials, which are costly, time-consuming, and require longer follow-up periods compared to studies using progression-free survival (PFS) as the primary endpoint. It is important to point out that PFS can provide evidence of the benefits of new therapies earlier because PFS cannot be affected by therapies after progression.

This study aimed to investigate the relationship between PFS and the type of surgery in our patients, as well as to analyze the impact of disease stage on long-term outcomes (3, 4).

MATERIALS AND METHODS

This scientific paper presents a retrospective study in which the following parameters were examined by analyzing the data obtained from the documentation of patients treated for ovarian cancer at the Department of Gynecology, Institute for Oncology and Radiology of Serbia (IORS) in Belgrade and the Institute of Vojvodina (IOV) in Sremska Kamenica: patient age, the period from the last chemotherapy to disease progression, the number of bevacizumab cycles received, disease stage, reasons for discontinuation of bevacizumab (disease progression/discontinuation due to complications), type of surgery, and pathological verification of the disease.

The patients were evaluated by the Tumor Oncology Board for Gynecological Tumors at IOV and IORS. The source of the material was archival data from IOV and IORS obtained through written records, Heliant, and the BIRPIS IOV system.

The study included patients over 18 years of age with histopathologically confirmed advanced ovarian cancer who were eligible for bevacizumab therapy. Patients with ovarian cancer without an indication for bevacizumab therapy or with concomitant other malignancies were excluded from the study. The patients were included in the study in the period from October 2017 to October 2020. The follow-up of the patients lasted 36 months. PFS is the time from the start of chemotherapy with bevacizumab to the onset of disease progression.

Descriptive statistical methods were applied in data analysis, including the calculation of measures of central tendency (arithmetic mean, median) and measures of dispersion (standard deviation). The Student's T-test was used to compare arithmetic means of parametric measurement values. The $\chi 2$ test was applied to assess statistical associations between the variables. A p-value of less than 0.05 was considered statistically significant. Survival was evaluated with Kaplan-Meier product-limit method. Median with corresponding 95% CI and Log-Rank test were used for progression-free survival (PFS) and overall survival (OS). Reported p-values were not corrected for multiple testing. Analyses were performed with Statistical Package for Social Sciences, Version 11.5 (SPSS, Inc., Chicago, IL, USA).

RESULTS

The study analyzed 111 patients treated with combined chemotherapy carboplatin/paclitaxel and biological therapy with bevacizumab. The full therapy with bevacizumab included 18 cycles.

The patients were between 36 and 84 years old, with an mean age of 60 years. Seventy-one patients completed the full bevacizumab therapy, representing 64% of the total number. In 37 patients, or 33.3%, therapy was discontinued due to disease progression. Adverse effects requiring therapy discontinuation occurred in 3 patients, accounting for 2.7% **(Table 1)**. In our study, two cases of deep vein thrombosis were observed, along with one case of grade 3 hypertension that was not adequately controlled with antihypertensive therapy.

Table 1. Treatment outcomes of patients

	n	%
Complete remission	71	64.0
Progression during therapy	37	33.3
Discontinuation due to therapy complications	3	2.7
Total	111	100.0

The minimum number of bevacizumab cycles administered was 4, the maximum was 18, with an mean of 15.3 ± 2.98 SD **(Table 2)**.

In all patients, an advanced stage of epithelial ovarian carcinoma was confirmed. According to the FIGO classification, 78 patients (70.3% of the total) were in stage IIIc, while the remaining 33 patients (29.7%) were in stage IV **(Table 3)**.

The patients were divided into two groups:

Group 1: Non-operated patients in whom pathological verification of the disease was obtained through core biopsy, exploratory surgery with biopsy, or aspiration of

Table 2. Number of administered bevacizumab cycles

	Mean	Standard deviation	Median	Minimum	Maximum
Number of bevacizumab cycles administered	15.3	2.98	11	4	18

ascites/pleural effusion. The total number of patients is 40 (36%) of all patients.

Group 2: Previously operated patients where cytoreductive surgery was performed. The total number of patients is 71 (64%) of all patients. These were suboptimal operated patients with residual disease after surgery **(Table 4)**.

Table 3. Distribution of FIGO stages among analyzed patients

Stage	n	%
IIIc	78	70.3
IV	33	29.7
Total number of ptc	111	100.0

Table 4. Operated vs. non-operated patients

	n	%
Non-operated patients	40	36.0
Operated patients	71	64.0
Total number of ptc	111	100.0

The median follow-up period was 30 months. The median overall progression-free survival (PFS) was 12.9 months (95% CI: 11.8- 13.9) for operated patients and 10.7 months (95% CI: 9.8-11.2) for non-operated patients. Using the Log-Rank, statistical significance (p =0.048) was demonstrated, indicating a statistically better PFS in the group of operated patients (suboptimal surgery) compared to those who were not operated **(Figure 1)**.

The median overall progression-free survival (PFS) for IIIc patients was 11.9 months (95% CI: 10.8- 12.3) and 10.9 months (95% CI: 10.2-11.2) for non-operated patients. There is no statistically significant difference in PFS between stages IIIc and IV, as determined by the Log-Rank, with p=0.261 **(Figure 2)**.

There was no statistically significant difference in the number of operated patients between disease stages IIIc and IV (p = 0.363) **(Figure 3)**.

Furthermore, no statistically significant difference was found in the treatment outcome with bevacizumab between non-operated and operated patients. Among the non-operated patients, 41% experienced progression during bevacizumab therapy, while in the operated (suboptimal) group, 30.4% experienced progression. A trend toward better out-

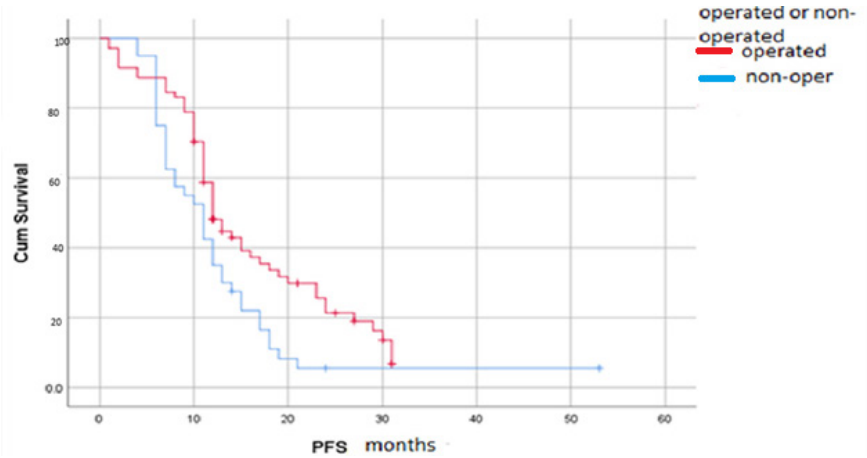

Figure 1. Kaplan-Meier Curves of PFS (Operated vs. Non-Operated Patients)

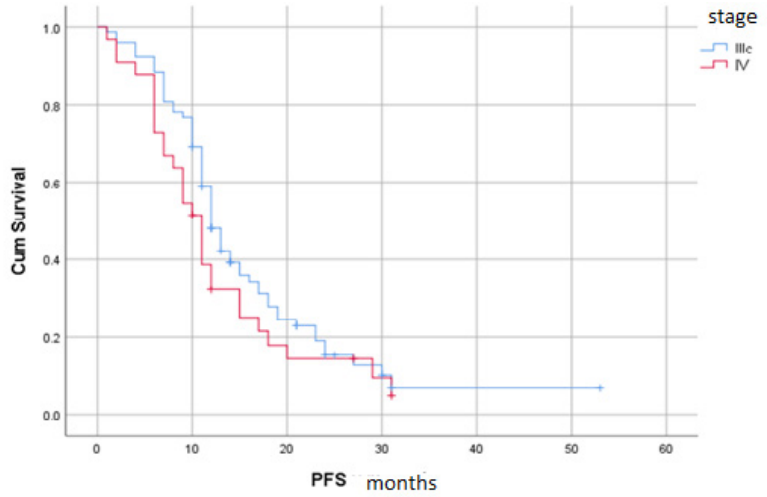

Figure 2. Kaplan-Meier Curve of PFS (FIGO IIIc vs. IV)

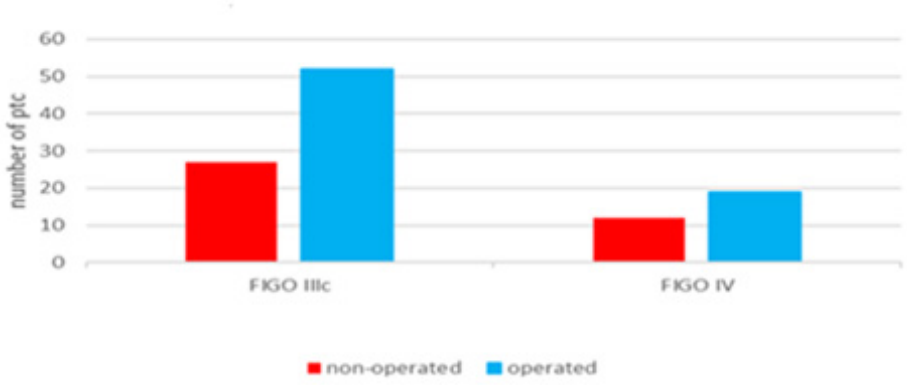

Figure 3. Number of operated patients by disease stage (IIIc vs. IV)

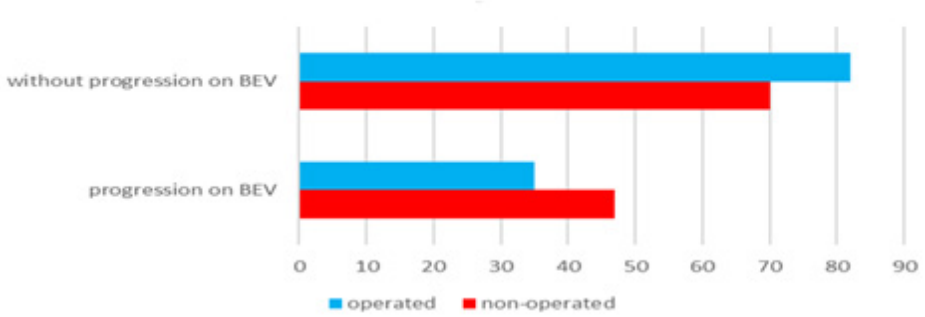

Figure 4. Progression during bevacizumab therapy

comes was observed in the operated group, though it did not reach statistical significance **(Figure 4)**.

There were no statistically significant differences in patient age between groups with different disease stages ($p = 0.201$), in disease outcome during the follow-up period (progression vs. no progression) ($p = 0.150$), or in the outcome of bevacizumab treatment (completion of 12-month therapy vs. discontinuation due to progression) ($p = 0.060$).

A statistically significant difference in patient age was observed between the operated and non-operated groups ($p = 0.037$). Operated patients were significantly younger (mean age 59.28 years) compared to non-operated patients (mean age 64.25 years).

DISCUSSION

In our retrospective analysis, we demonstrated the importance and statistical significance of surgical extensiveness for long-term outcomes, showing that progression-free survival (PFS) was significantly better in patients who underwent suboptimal surgery compared to those who did not have surgery. This aligns with the current literature, where the standard management of advanced epithelial ovarian cancer involves correct surgical staging and optimal tumor cytoreduction, followed by chemotherapy with platinum and taxane-based agents. Standard surgical staging includes peritoneal washings, total hysterectomy, bilateral salpingo-oophorectomy, inspection of all abdominal organs and the peritoneal surface, biopsies of suspicious or random areas, omentectomy, and para-aortic lymphadenectomy. Achieving complete tumor cytoreduction has been shown to improve survival (5). Importantly, optimal surgical cytoreduction is one of the strongest predictors of outcomes in patients with high-grade serous carcinoma (HGSOC) treated with primary cytoreductive surgery (6, 7).

Our results indicated that there is no statistically significant difference in long-term outcomes between stages IIIc and IV, which aligns with previous data. This correlation between progression-free survival (PFS) and overall survival (OS) following primary treatment for ovarian cancer underscores the validity of using PFS as a primary endpoint. Most of the data come from observational studies, which have limited information on disease stage and histology. Patients with advanced stage (III or IV) ovarian cancer generally have a poor prognosis. The standard treatment options of surgery and chemotherapy extend survival beyond diagnosis for five years or more in only about 45% of patients, with little difference observed between stage IIIc with residual disease and stage IV (2, 3). There was no statistically significant difference in the number of patients who underwent surgery based on the disease stage, whether stage IIIc or IV, which aligns with previous data where comparison of surgical extensiveness revealed no difference in tumor stage between IIIc and IV (8).

Additionally, no statistically significant difference was found in the treatment outcomes with bevacizumab between operated and non-operated patients. Although there was a trend towards better outcomes in the operated group, it was not sufficient to reach statistical significance.

Several studies have demonstrated that women with advanced high-grade serous carcinoma (HGSOC) who underwent primary cytoreductive surgery (PCS) had better survival outcomes compared to those who received neoadjuvant chemotherapy (NAC) followed by interval surgery, regardless of the number of NAC cycles administered. Furthermore, achieving optimal cytoreduction did not confer a survival advantage in the NAC group, whereas patients who underwent primary surgery exhibited a clear survival benefit (9, 10). Overall, the use of neoadjuvant chemotherapy as a first-line treatment for women with newly diagnosed HGSOC remains controversial (10).

A statistically significant difference in the age of patients was found between the operated and non-operated groups. Operated patients were significantly younger (mean age 59.28 years) compared to non-operated patients. Patients treated with neoadjuvant chemotherapy were significantly older than patients treated with PCS according to the results of Stewart's Canadian study (8).

Studies have shown that operative treatment has an impact on all prognostic groups in all FIGO stages of the disease, regardless of the different preoperative tumor burden (10). Of course, tumor biology itself remains one of the most important prognostic factors contributing to the poor outcome of patients. A meta-analysis including 6885 patients with stage III and IV ovarian cancer and examining the effect of surgery on PFS showed that overall cytoreduction affects survival (12, 13).

CONCLUSION

Our data indicate that surgical factors, such as the extent of surgery and the rate of optimal cytoreduction, appear to confer a survival advantage for women with ovarian cancer treated with a combination of bevacizumab and chemotherapy. New studies will show whether the stage of the disease plays a significant role in the survival of patients with ovarian cancer, since stages IIIc and IV represent advanced disseminated disease with almost equally poor outcomes. Also, the dilemma remains whether the significantly higher percentage of operated on younger patients is due to the willingness of the surgeon to operate and the lower risk of complications from such procedures.

Author contributions

The conception or design of the work: SR, MMK, and SSR.
The acquisition, analysis, or interpretation of data: MMK, SM and MM.
Preparing the draft of the manuscript or interpretation of revised version of manuscript SR.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209–49.
2. Gupta S, Nag S, Aggarwal S, Rauthan A, Warrier N. Maintenance therapy for recurrent epithelial ovarian cancer: Current therapies and future perspectives - a review. J Ovarian Res 2019; 12: 103.
3. Oza AM, Castonguay V, Tsoref D, Diaz-Padilla I, Karakasis K, Mackay H, et al. Progression-free survival in advanced ovarian cancer: a Canadian review and expert panel perspective. Curr Oncol 2011; 18 (Suppl 2):S20–7.
4. Sjoquist KM, Lord SJ, Friedlander ML, John Simes R, Marschner IC, Lee CK. Progression-free survival as a Surrogate Endpoint for overall survival in modern ovarian cancer trials: a meta-analysis. Ther Adv Med Oncol 2018; 10: 1758835918788500.
5. Martín-Cameán M, Delgado-Sánchez E, Piñera A, Diestro MD, De Santiago J, Zapardiel I. The role of surgery in advanced epithelial ovarian cancer. Ecancermedicalscience 2016; 10: 666.
6. Bian C, Yao K, Li L, Yi T, Zhao X. Primary debulking surgery vs. neoadjuvant chemotherapy followed by interval debulking surgery for patients with advanced ovarian cancer. Arch Gynecol Obstet. 2016; 293(1): 163-168.
7. Mogi K, Yoshihara M, Emoto R, Miyamoto E, Fujimoto H, Uno K, et al. Histology-specific long-term oncologic outcomes in patients with epithelial ovarian cancer who underwent complete tumor resection: The implication of occult seeds after initial surgery. PLoS One 2024; 19(11): e0311421.
8. Stewart JM, Tone AA, Jiang H, Bernardini MQ, Ferguson S, Laframboise S,et al. The optimal time for surgery in women with serous ovarian cancer. Can J Surg . 2016; 59(4): 223–232.
9. Manning-Geist BL, Hicks-Courant K, Gockley AA, Clark RM, Del Carmen MG, Growdon WB et al. "Moving beyond "complete surgical resection" and "optimal": is low-volume residual disease another option for primary Debulking surgery". Gynecol Oncol 2018; 150: 233–8.
10. Melamed A, Manning-Geist B, Bregar AJ, Diver EJ, Goodman A, Del Carmen MG, et al. Associations between residual disease and survival in epithelial ovarian cancer by histologic type. Gynecol Oncol 2017; 147: 250–6.
11. du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer 2009; 115(6): 1234-44.
12. Bristow RE, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol 2002; 20: 1248–59.
13. Manning-Geist BL, Hicks-Courant K, Gockley AA, Clark RM, Del Carmen MG, Growdon WB, et al. A novel classification of residual disease after interval debulking surgery for advanced-stage ovarian cancer to better distinguish oncologic outcome. Am J Obstet Gynecol 2019; 221(4): 326.e1-326.e7.

UTICAJ STADIJUMA BOLESTI I HIRURGIJE NA DUGOROČNE ISHODE KOD PACIJENTKINJA SA KARCINOMOM JAJNIKA

Marijana Milović-Kovačević[1, 2], Mladen Marinković[1, 2], Slobodan Maričić[3], Suzana Stojanović Rundić[1, 2], Sandra Radenković[1]

Sažetak

Uvod: Standardna terapija za pacijente sa karcinomom jajnika uključuje citoreduktivnu operaciju praćenu hemoterapijom na bazi platine. Faktori kao što su stadijum bolesti pri postavljanju dijagnoze, histološki tip tumora, veličina tumora i prisustvo rezidualne bolesti posle citoreduktivne operacije utiču na prognozu pacijenata.

Materijal i metode: Ovaj naučni rad predstavlja retrospektivnu studiju u kojoj su analizirani sledeći parametri analizom podataka dobijenih iz dokumentacije pacijenata lečenih od karcinoma jajnika na Institutu za onkologiju i radiologiju Srbije i Institutu za onkologiju Vojvodine: godine starosti pacijenata, period od poslednje hemoterapije do progresije bolesti, broj primljenih ciklusa bevacizumaba, stadijum bolesti, razlozi za prekid bevacizumaba, vrsta hirurgija i patološka verifikacija bolesti.

Rezultati: Pokazali smo da je PFS duži u operisanoj grupi (suboptimalna operacija) u odnosu na one koji nisu operisani ($p < 0,05$, Log-Rank test). Ne postoji statistički značajna razlika u PFS između stadijuma IIIc i IV, što je utvrđeno Log-Rank testom. Takođe, u našem istraživanju nije bilo značajne razlike u broju operisanih pacijenata na osnovu IIIc ili IV stadijuma bolesti ($p = 0, 361$).

Zaključci: Naši podaci pokazuju da se čini da citoredukcija daje prednost u preživljavanju kod žena sa karcinomom jajnika lečenih kombinacijom bevacizumaba i hemoterapije. Nove studije treba da pokažu da li stadijum bolesti igra značajnu ulogu u preživljavanju pacijenata sa karcinomom jajnika.

Ključne reči: karcinom jajnika, preživljavanje bez progresije bolesti, tip operacije, stadijum bolesti

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REVIEW ARTICLE

Neuromyelitis Optica spectrum disorders: therapeutic considerations

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Summary

Neuromyelitis Optica spectrum disorder (NMOSD) is a rare but debilitating autoimmune disease of the central nervous system (CNS) for which several biological therapies have been approved recently. Historically, NMOSD disease-modifying treatments relied on wide-spectrum off-label conventioanl immunosuppressants, such as azathioprine, and mycophenolate mofetil. Since 2015, evidence has accumulated to support off-label biological therapy (rituximab) and to approve satralizumab, inebilizumab, eculizumab, and ravulizumab. This next generation of drugs provides several targeted disease-modifying treatment options for NMOSD. Here, we first review the mechanistic rationales associated with their specific targets. Then we review the pivotal evidence supporting their use in practice. The current therapeutic options in NMOSD comprise three targeted mechanisms at different stages of a unique tissue-injury cascade: B-cell depleting, anti-cytokine, and anti-complement therapies. One drug from each class has been approved for market release. The current consensus proposes positioning the approved drugs as first-line treatments for newly diagnosed patients and as alternative therapies in case of failure of historical treatment.

Keywords: Neuromyelitis Optica spectrum disorder, treatment, disease-modifying treatment

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INTRODUCTION

Neuromyelitis Optica spectrum disorder (NMOSD) is an immune-mediated disease of the central nervous system (CNS), which is predominantly manifested by the appearance of optic neuritis (ON), transverse myelitis (TM), but also by the involvement of other CNS structures such as diencephalon, brainstem and area postrema (1). The main substrate of etiopathogenesis is related to the auto-antibodies, immunoglobulins G class, directed towards the transmembrane water pore of aquaporin-4 (AQP4-IgG) (1-3). AQP4 is expressed on the astrocytes, especially on the foot-like extensions of astrocytes, and it plays a major role in regulating the flow of water molecules through the cell membranes (4, 5). It has been demonstrated that about 80% of people with NMOSD have AQP4-IgG in their blood, which makes these autoantibodies an important molecular biomarker of the disease, based on which currently valid diagnostic criteria define seropositive and seronegative forms of NMOSD (1-3, 6).

The pathophysiology of seropositive NMOSD is driven by antibody-mediated humoral and cellular immune activation, leading to astrocyte destruction through mechanisms such as complement-dependent cytotoxicity and antibody-induced cellular cytotoxicity. Additionally, the inflammatory milieu contributes to the damage of adjacent CNS cells (7-11). Etiopathogenetic and pathophysiological mechanisms of the seronegative NMOSD have not been fully elucidated. Several various potential explanations for the occurrence of the seronegative NMOSD have been proposed, such as the existence of certain other autoantibodies, lower sensitivity of available tests, cellular mechanisms of pathogenesis, etc. (7, 12-14). The clinical course of the majority of NMOSD cases is recurrent and characterized by unpredictable and potentially very severe relapses that contribute to the development of permanent disability in the affected individuals (10, 12, 15). It has been shown that AQP4-IgG seropositivity in NMOSD patients increases the risk of other comorbid autoimmune diseases (16). In addition to the relapses, patients with NMOSD are burdened with numerous manifestations of the dysfunction of the autonomic nervous system, depression, anxiety and fatigue, which significantly affect the quality of life of these individuals (17-19).

The therapeutic approach to NMOSD is based on the current knowledge related to the pathophysiology and clinical characteristics of the disease and have two main goals: therapy of the relapse and long-term prevention of new relapses (10, 15, 20).

The aim of acute relapse therapy is a better and faster recovery of the neurological deficit (15, 21, 22). Following the latest recommendations, relapse therapy in both AQP4-IgG seropositive and seronegative NMOSD patients, comprise administration of corticosteroids (glucocorticoids) and/or blood apheresis according to established protocols as early as possible (20). Medicines used in chronic therapy to prevent relapse in NMOSD include:

conventional immunosuppressive drugs, as well as recently approved, evidence-based, specific therapies, and various monoclonal antibodies (20).

CONVENTIONAL IMMUNOSUPPRESSIVE THERAPIES

Drugs that achieve non-specific immunosuppression, such as azathioprine, and mycophenolate mofetil, potentially in combination with oral glucocorticoids, have retained their role in NMOSD therapy regardless of serostatus (20). For years, these drugs have been the primary treatment for all forms of NMOSD to prevent new relapses. However, their use has been limited by the risk of numerous side effects, particularly long-term administration (20, 23, 24). Under current conditions, glucocorticoids are primarily used as adjunctive therapy for preventing new NMOSD relapses (20). In certain areas of the world, where no other drugs are available for the treatment of NMOSD, glucocorticoids are still used as chronic monotherapy for this disease (20). Glucocorticoids achieve their effect even in lower doses that are taken chronically, but they can often induce the occurrence of adverse events, such as lymphopenia and hepatotoxicity (20). Additionally, long-term use of glucocorticoids is associated with more frequent occurrences of diabetes mellitus, hypertension, osteoporosis, and other disorders (20, 24). Recent recommendations suggest oral glucocorticoids should not be used as monotherapy in the prevention of NMOSD relapse, except when no other therapeutic options are available (20).

Azathioprine is a drug that is used in the treatment of NMOSD in a dose of 2.5-3 mg/kg/day with full therapeutic effect achieved after 6-12 months. It is recommended to overlap it during the first six months with oral glucocorticoids, which quickly achieve their effect, thus bridging the therapeutic gap (20, 25, 26). Frequent adverse effects of azathioprine refer to the occurrence of lymphopenia, thrombocytopenia, hepatotoxicity, gastrointestinal disturbances, and long-term effects that may lead to the potential occurrence of malignancy and secondary infections (20, 23, 25, 27). Studies have shown that lymphopenia occurs in about 13% of NMOSD patients treated with azathioprine (28). Although the frequency of secondary infections in patients treated with azathioprine varies, the incidence of infections is not high (27). However, very rare cases of progressive multifocal leukoencephalopathy (PML) have been described in patients treated exclusively with azathioprine (29, 30). In general, since all side effects are mainly related to the length of treatment and the dose of azathioprine, dose reduction or temporary discontinuation of the drug can alleviate these side effects (27).

Mycophenolate mofetil in therapeutic doses of 1000 to 2000 mg/day has a similar effectiveness and side effect profile to azathioprine (20, 27). The time required to achieve a full therapeutic effect is shorter compared to azathioprine and amounts to 6-12 weeks (20, 31). The most common side effects of mycophenolate mofetil are: leukopenia with secondary infections, vomiting, and diarrhea (27). In a meta-analysis that included 11 studies of patients with NMOSD treated with mycophenolate mofetil, it was shown that side effects were present in 17.8%, while individual studies reported the frequency of side effects in up to 43% (27, 32, 33).

MONOCLONAL ANTIBODIES

Recently, several prospective randomized controlled trials (RCT) have led to FDA approval of the first three immunotherapies for patients with AQP4-IgG-positive NMOSD: eculizumab in June 2019, inebilizumab in June 2020, and satralizumab in August 2020 (34-37). In addition, rituximab was approved for NMOSD in Japan in June 2022 based on the results of an investigator-initiated phase II/III clinical study (38), and in May 2023, the EMA approved ravulizumab for the treatment of AQP4-IgG-positive NMOSD.

Satralizumab

Satralizumab is a humanized monoclonal antibody against interleukin-6 receptor (IL-6R) (20). Satralizumab was approved for the treatment of AQP4-IgG seropositive NMOSD in adult patients and adolescents (aged 12 and older) in 2020 in the USA and in 2021 in Europe (20, 39, 40). Satralizumab reduces the relapse rate by over 70% during a follow-up period of more than 4 years (41). In more than 50% of patients with NMOSD, serum antibodies against Satralizumab were detected, but their clinical significance is unknown (20). Possible side effects of the drug are related to laboratory parameters such as neutropenia, thrombocytopenia, and side effects in the form of infusion reactions, headache, and arthralgia, while no serious opportunistic infections have been reported until now (20, 27).

Rituximab

Rituximab is a monoclonal antibody against a cluster of differentiation (CD) 20 molecules on the surface of B lymphocytes, causing depletion of these cells (20, 43). The full therapeutic effect of Rituximab is achieved in 8-12 weeks, which is why the initial introduction of oral is advised glucocorticoids in the first few months (20). Rituximab is the only monoclonal antibody that has shown efficacy in the treatment of seropositive and seronegative NMOSD, which is of great importance (20, 43). Rituximab achieves a reduction in the relapse rate in NMOSD by over 80% (20). A potential reason for the absence of a positive therapeutic response is the possible occurrence of serum-neutralizing antibodies against Rituximab, which occurs in a different percentage of treated patients (44, 45). The main side effects of Rituximab therapy are headache, nausea, infections, and infusion reactions (20, 27). The most common side effects of Rituximab in patients with NMOSD are infusion reactions 10-13%, followed by infections mainly of the respiratory and urinary tract, 9% (27, 46, 47).

Inebilizumab

Inebilizumab is a humanized monoclonal antibody that causes depletion of the CD19 subpopulation of B lymphocytes (20). The drug was approved in 2020 in the USA, and in 2022 in Europe as a therapy for seropositive NMOSD (20, 48). It is most likely that inebilizumab achieves its full effect within 6-8 weeks of starting therapy (20). Inebilizumab caused a significant reduction in the relapse rate in NMOSD patients over time, with the most frequent occurrence of relapse occurring only during the first year of follow-up (49). The most common adverse effects of inebilizumab are related to arthralgia and back pain, headache, and infusion reactions (20, 48). There may also be a slightly higher risk of infections, among which, according to the findings of certain studies, urinary infections are the most common, accounting for up to 20% (27). So far, no cases of severe opportunistic infections have been reported, although a case of potential PML has been described, for which, to the best of our knowledge, this diagnosis has not been confirmed with certainty (20, 27, 48).

Eculizumab

Eculizumab is a humanized monoclonal antibody directed against the C5 complement component, blocking the cascade reaction of the complement system in the pathogenesis of NMOSD (10, 20). Eculizumab was approved for the treatment of seropositive NMOSD in 2019 in the USA, while in Europe, it was approved for relapsing forms of seropositive NMOSD (50-52). Eculizumab achieves its effect very quickly after application by strongly blocking the activity of the C5 component of the complement (20, 51). Eculizumab has shown remarkable efficacy over a follow-up period of just over a year, with complete relapse control in patients with NMOSD (20, 53). One of the most common side effects of eculizumab is headache, while back pain, diarrhea, and nausea occur less frequently (27). Notably, eculizumab increases the risk of infections caused by bacteria from the genus Meningococcus and other encapsulated bacteria regardless of prior vaccination. It also heightens the risk of certain fungal infections (20, 27, 52, 54).

Ravulizumab

Ravulizumab is a monoclonal antibody that achieves its effect in treating NMOSD by inhibiting the C5 complement component (20). Ravulizumab also potently and rapidly inhibits the cascade reaction of the complement system (20). Ravulizumab is a very effective drug in preventing the occurrence of relapse in the seropositive form of NMOSD with a complete, 100% cessation of relapse during a one-year follow-up (20, 55). Ravulizumab and eculizumab, both targeting the complement system, have similar molecular structures, as well as comparable therapeutic and safety profiles (20). Adverse effects of Ravulizumab include headache, anemia, leukopenia, as well as a tendency to respiratory infections as well as meningococcal and fungal infections (20).

RECOMMENDATIONS AND MODALITIES OF LONG-TERM THERAPY

Recently published recommendations for pharmacological therapy of NMOSD suggest different treatment modalities for AQP4-IgG seropositive and seronegative NMOSD (20). For the prevention of relapse in the seropositive form of NMOSD, all the above-mentioned drugs can be used, depending on the condition, age, and preferences of the patient, comorbidities, as well as characteristics of the disease itself, such as the frequency and severity of relapse and socioeconomic circumstances (20). Potent monoclonal antibodies are recommended as first-line monotherapy to prevent NMOSD relapses (20). These recommendations suggest that the first line of therapy in seronegative NMOSD should be drugs from the group of conventional immunosuppressive drugs or rituximab monotherapy (20). If there is no therapeutic effect, conventional immunosuppressive drugs should be switched to rituximab (20).

Females with NMOSD in the reproductive period should plan pregnancy in consultation with a neurologist in the phases of remission of the disease. Additionally, the chronic administration of drugs to control the relapse of the disease should not be interrupted or delayed (20, 56). Methotrexate and mycophenolate mofetil are teratogenic and should be avoided in women of reproductive age, as well as during pregnancy and breastfeeding (20, 57).

Azathioprine or monoclonal antibodies could be used in pregnancy, when necessary, with careful consideration of each drug's profile and all clinical characteristics of the individual patient (20, 57). When using these drugs, special monitoring by neurologists, gynecologists/obstetricians, and other members of the medical team is necessary (20, 57).

OTHER TREATMENT MODALITIES

Administration of intravenous immunoglobulins (IVIG), 1g/kg for 4 weeks, showed positive effects in relapse control in NMOSD in children and adults (20). The combination of IVIG with conventional immunosuppressive drugs, such as azathioprine, can have a positive effect on the prevention of relapse in NMOSD (20). Methotrexate may also have a role in relapse prevention therapy, particularly in individuals with autoimmune comorbidities and NMOSD (20, 58, 59). The application of a combination of intermittent apheresis (TIP) with conventional immunosuppressive drugs can be a form of treatment when other options are not available or not applicable (20, 58, 59).

CONCLUSION

The existence of a molecular biomarker and clearly defined diagnostic criteria enables a quick and accurate diagnosis of NMOSD. On the other hand, knowledge regarding the pathophysiological mechanisms underlying different forms of NMOSD enables the design of goal-directed new therapies, which support precision medicine and emphasize the importance of an individual approach. Biological therapy represents an important step in the prevention of relapse in NMOSD via using monoclonal antibodies, which reduce the deleterious effect of this disease on the degree of disability, quality of life, and prognosis of the disease. Further research is necessary in order to find potential therapeutic targets in the seronegative NMOSD, as well as the development of new and safer therapeutic agents and treatment modalities for all forms of NMOSD.

Author Contributions

MA, SM, NV, OT, MB, and JD conceived and wrote the paper, revised it for important intellectual content, and approved the final submission.

REFERENCES

1. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 2015; 85(2): 177-89.
2. Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet 2004; 364(9451): 2106-12.
3. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 2005; 202(4): 473-7.
4. Wolburg H, Wolburg-Buchholz K, Fallier-Becker P, Noell S, Mack AF. Structure and functions of aquaporin-4-based orthogonal arrays of particles. Int Rev Cell Mol Biol 2011; 287: 1-41.

5. Dermietzel R. Visualization by freeze-fracturing of regular structures in glial cell membranes. Naturwissenschaften 1973; 60(4): 208.
6. Dujmovic I, Mader S, Schanda K, Deisenhammer F, Stojsavljevic N, Kostic J, et al. Temporal dynamics of cerebrospinal fluid anti-aquaporin-4 antibodies in patients with neuromyelitis optica spectrum disorders. J Neuroimmunol 2011; 234(1-2): 124-30.
7. Chang VTW, Chang HM. Review: Recent advances in the understanding of the pathophysiology of neuromyelitis optica spectrum disorder. Neuropathol Appl Neurobiol 2020; 46(3): 199-218.
8. Carnero Contentti E, Correale J. Neuromyelitis optica spectrum disorders: from pathophysiology to therapeutic strategies. J Neuroinflammation 2021; 18(1): 208.
9. Ratelade J, Asavapanumas N, Ritchie AM, Wemlinger S, Bennett JL, Verkman AS. Involvement of antibody-dependent cell-mediated cytotoxicity in inflammatory demyelination in a mouse model of neuromyelitis optica. Acta Neuropathol 2013; 126(5): 699-709.
10. Jarius S, Aktas O, Ayzenberg I, Bellmann-Strobl J, Berthele A, Giglhuber K, et al. Update on the diagnosis and treatment of neuromyelits optica spectrum disorders (NMOSD) - revised recommendations of the Neuromyelitis Optica Study Group (NEMOS). Part I: Diagnosis and differential diagnosis. J Neurol 2023; 270(7): 3341-68.
11. Duan T, Smith AJ, Verkman AS. Complement-dependent bystander injury to neurons in AQP4-IgG seropositive neuromyelitis optica. J Neuroinflammation 2018; 15(1): 294.
12. Jarius S, Ruprecht K, Wildemann B, Kuempfel T, Ringelstein M, Geis C, et al. Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: A multicentre study of 175 patients. J Neuroinflammation 2012; 9: 14.
13. Wu Y, Geraldes R, Juryńczyk M, Palace J. Double-negative neuromyelitis optica spectrum disorder. Mult Scler 2023; 29(11-12): 1353-62.
14. Sato DK, Callegaro D, Lana-Peixoto MA, Nakashima I, Fujihara K. Seronegative Neuromyelitis Optica Spectrum--the challenges on disease definition and pathogenesis. Arq Neuropsiquiatr 2014; 72(6): 445-50.
15. Kleiter I, Gahlen A, Borisow N, Fischer K, Wernecke KD, Wegner B, et al. Neuromyelitis optica: Evaluation of 871 attacks and 1,153 treatment courses. Ann Neurol 2016; 79(2): 206-16.
16. Pekmezovic T, Jovicevic V, Andabaka M, Momcilovic N, Veselinovic N, Tamas O, et al. Aquaporin4-IgG seropositivity significantly increases the risk of comorbid autoimmune diseases in NMOSD patients: population-based registry data. J Neurol 2024; 271(12): 7525-7536.
17. Crnošija L, Krbot Skorić M, Andabaka M, Junaković A, Martinović V, Ivanović J, et al. Autonomic dysfunction in people with neuromyelitis optica spectrum disorders. Mult Scler 2020; 26(6): 688-695.
18. Habek M, Andabaka M, Fanciulli A, Brecl Jakob G, Drulović J, et al. Sudomotor dysfunction in people with neuromyelitis optica spectrum disorders. Eur J Neurol 2022; 29(9): 2772-2780.
19. Andabaka M, Pekmezovic T, Crnosija L, Veselinovic N, Junakovic A, Tamas O, et al. Impact of the autonomic dysfunction on the quality of life in people with NMOSD and MS: An international cross-sectional study. Mult Scler Relat Disord 2023; 79: 104953.
20. Kümpfel T, Giglhuber K, Aktas O, Ayzenberg I, Bellmann-Strobl J, Häußler V, et al. Update on the diagnosis and treatment of neuromyelitis optica spectrum disorders (NMOSD) - revised recommendations of the Neuromyelitis Optica Study Group (NEMOS). Part II: Attack therapy and long-term management. J Neurol 2024; 271(1): 141-76.
21. Abboud H, Petrak A, Mealy M, Sasidharan S, Siddique L, Levy M. Treatment of acute relapses in neuromyelitis optica: Steroids alone versus steroids plus plasma exchange. Mult Scler 2016; 22(2): 185-92.
22. Akaishi T, Takeshita T, Himori N, Takahashi T, Misu T, Ogawa R, et al. Rapid Administration of High-Dose Intravenous Methylprednisolone Improves Visual Outcomes After Optic Neuritis in Patients With AQP4-IgG-Positive NMOSD. Front Neurol 2020; 11: 932.
23. Lebrun C, Rocher F. Cancer Risk in Patients with Multiple Sclerosis: Potential Impact of Disease-Modifying Drugs. CNS Drugs 2018; 32(10): 939-49.
24. Oray M, Abu Samra K, Ebrahimiadib N, Meese H, Foster CS. Long-term side effects of glucocorticoids. Expert Opin Drug Saf 2016; 15(4): 457-65.
25. Confavreux C, Saddier P, Grimaud J, Moreau T, Adeleine P, Aimard G. Risk of cancer from azathioprine therapy in multiple sclerosis: a case-control study. Neurology 1996; 46(6): 1607-12.
26. Costanzi C, Matiello M, Lucchinetti CF, Weinshenker BG, Pittock SJ, Mandrekar J, et al. Azathioprine: tolerability, efficacy, and predictors of benefit in neuromyelitis optica. Neurology 2011; 77(7): 659-66.
27. Giglhuber K, Berthele A. Adverse Events in NMOSD Therapy. Int J Mol Sci 2022; 23(8): 4154.
28. Luo D, Wei R, Tian X, Chen C, Ma L, Li M, et al. Efficacy and safety of Azathioprine for neuromyelitis optica spectrum disorders: A meta-analysis of real-world studies. Mult Scler Relat Disord 2020: 46: 102484.
29. Schmedt N, Andersohn F, Garbe E. Signals of progressive multifocal leukoencephalopathy for immunosuppressants: A disproportionality analysis of spontaneous reports within the US Adverse Event Reporting System (AERS). Pharmacoepidemiol Drug Saf 2012; 21: 1216-1220.
30. Flanagan E.P, Aksamit A.J, Kumar N, Morparia N.P, Keegan B.M, Weinshenker B.G. Simultaneous PML-IRIS and myelitis in a patient with neuromyelitis optica spectrum disorder. Neurol Clin Pract 2013; 3: 448-451.
31. Montcuquet A, Collongues N, Papeix C, Zephir H, Audoin B, Laplaud D, et al. Effectiveness of mycophenolate mofetil as first-line therapy in AQP4-IgG, MOG-IgG, and seronegative neuromyelitis optica spectrum disorders. Mult Scler 2017; 23(10): 1377-84.
32. Songwisit S, Kosiyakul P, Jitprapaikulsan J, Prayoonwiwat N, Ungprasert P, Siritho S. Efficacy and safety of mycophenolate mofetil therapy in neuromyelitis optica spectrum disorders: A systematic review and meta-analysis. Sci Rep 2020; 10: 16727.
33. Huang Q, Wang J, Zhou Y, Yang H, Wang Z, Yan Z, et al. Low-Dose Mycophenolate Mofetil for Treatment of Neuromyelitis Optica Spectrum Disorders: A Prospective Multicenter Study in South China. Front Immunol 2018; 9: 2066.
34. Cree BAC, Bennett JL, Kim HJ, Weinshenker BG, Pittock SJ, Wingerchuk DM, Fujihara K, Paul F, Cutter GR, Marignier R, Green AJ, Aktas O, Hartung H-P, Lublin FD, Drappa J, Barron G, Madani S, Ratchford JN, She D, Cimbora D, Katz E. Inebilizumab for the treatment of neuromyelitis optica spectrum disorder (N-MOmentum): a double-blind, randomised placebo-controlled phase 2/3 trial. The Lancet 2019; 394: 1352-1363.
35. Pittock SJ, Berthele A, Fujihara K, Kim HJ, Levy M, Palace J, Nakashima I, Terzi M, Totolyan N, Viswanathan S, Wang KC, Pace A, Fujita KP, Armstrong R, Wingerchuk DM. Eculizumab in aquaporin-4-positive neuromyelitis optica spectrum disorder. N Engl J Med 2019; 381: 614-625
36. Traboulsee A, Greenberg BM, Bennett JL, Szczechowski L, Fox E, Shkrobot S, Yamamura T, Terada Y, Kawata Y, Wright P, Gianella-Borradori A, Garren H, Weinshenker BG. Safety and efficacy of satralizumab monotherapy in neuromyelitis optica spectrum disorder: a randomised, double-blind, multicentre, placebo-controlled phase 3 trial. Lancet Neurol 2020; 19: 402-412.
37. Yamamura T, Kleiter I, Fujihara K, Palace J, Greenberg B, Zakrzewska-Pniewska B, Patti F, Tsai CP, Saiz A, Yamazaki H, Kawata Y, Wright P, De Seze J. Trial of satralizumab in neuromyelitis optica spectrum disorder. N Engl J Med 2019; 381: 2114-2124.
38. Tahara M, Oeda T, Okada K, Kiriyama T, Ochi K, Maruyama H, Fukaura H, Nomura K, Shimizu Y, Mori M, Nakashima I, Misu T, Umemura A, Yamamoto K, Sawada H. Safety and efficacy of rituximab in neuromyelitis optica spectrum disorders (RIN-1 study): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2020; 19: 298-306.
39. Chihara N, Aranami T, Sato W, Miyazaki Y, Miyake S, Okamoto T, et al. Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica. Proc Natl Acad Sci USA 2011; 108(9): 3701-6.

40. Traboulsee A, Greenberg BM, Bennett JL, Szczechowski L, Fox E, Shkrobot S, et al. Safety and efficacy of satralizumab monotherapy in neuromyelitis optica spectrum disorder: a randomised, double-blind, multicentre, placebo-controlled phase 3 trial. Lancet Neurol 2020; 19(5): 402-12.
41. Yamamura T, Kleiter I, Fujihara K, Palace J, Greenberg B, Zakrzewska-Pniewska B, et al. Trial of Satralizumab in Neuromyelitis Optica Spectrum Disorder. N Engl J Med 2019; 381(22): 2114-24.
42. Kleiter I, Traboulsee A, Palace J, Yamamura T, Fujihara K, Saiz A, et al. Long-term Efficacy of Satralizumab in AQP4-IgG-Seropositive Neuromyelitis Optica Spectrum Disorder From SAkuraSky and SAkuraStar. Neurol Neuroimmunol Neuroinflamm 2023; 10(1):
43. Tahara M, Oeda T, Okada K, Kiriyama T, Ochi K, Maruyama H, et al. Safety and efficacy of rituximab in neuromyelitis optica spectrum disorders (RIN-1 study): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2020; 19(4): 298-306.
44. Oomen I, Nassar-Sheikh Rashid A, Bouts AHM, Gouw SC, Kuijpers TW, Rispens T, et al. Anti-rituximab antibodies affect pharmacokinetics and pharmacodynamics of rituximab in children with immune-mediated diseases. Clin Exp Rheumatol 2022; 40(1): 183-90.
45. Li T, Zhang LJ, Zhang QX, Yang CS, Zhang C, Li YJ, et al. Anti-Rituximab antibody in patients with NMOSDs treated with low dose Rituximab. J Neuroimmunol 2018; 316: 107-11.
46. Damato V, Evoli A, Iorio R. Efficacy and Safety of Rituximab Therapy in Neuromyelitis Optica Spectrum Disorders: A Systematic Review and Meta-analysis. JAMA Neurol 2016; 73: 1342-1348.
47. Nikoo Z, Badihian S, Shaygannejad V, Asgari N, Ashtari F. Comparison of the efficacy of Azathioprine and Rituximab in neuromyelitis optica spectrum disorder: A randomised clinical trial. J Neurol 2017; 264: 2003-2009.
48. Cree BAC, Bennett JL, Kim HJ, Weinshenker BG, Pittock SJ, Wingerchuk DM, et al. Inebilizumab for the treatment of neuromyelitis optica spectrum disorder (N-MOmentum): a double-blind, randomised placebo-controlled phase 2/3 trial. Lancet 2019; 394(10206): 1352-63.
49. Rensel M, Zabeti A, Mealy MA, Cimbora D, She D, Drappa J, et al. Long-term efficacy and safety of inebilizumab in neuromyelitis optica spectrum disorder: analysis of aquaporin-4– immunoglobulin G–seropositive participants taking inebilizumabfor.4 years in the N-MOmentum trial. Mult Scler 2022; 28: 925-932.
50. Pittock SJ, Berthele A, Fujihara K, Kim HJ, Levy M, Palace J, et al. Eculizumab in Aquaporin-4-Positive Neuromyelitis Optica Spectrum Disorder. N Engl J Med 2019; 381(7): 614-25.
51. Pittock SJ, Fujihara K, Palace J, Berthele A, Kim HJ, Oreja-Guevara C, et al. Eculizumab monotherapy for NMOSD: Data from PREVENT and its open-label extension. Mult Scler 2022; 28(3): 480-6.
52. Pittock SJ, Lennon VA, McKeon A, Mandrekar J, Weinshenker BG, Lucchinetti CF, et al. Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study. Lancet Neurol 2013; 12(6): 554-62.
53. Ringelstein M (2022) ECTRIMS 2022 - ePoster. Mult Scler J 2022; 28: 692-945.
54. McNamara L.A, Topaz N, Wang X, Hariri S, Fox L.A, MacNeil J.R. High Risk for Invasive Meningococcal Disease Among Patients Receiving Eculizumab (Soliris) Despite Receipt of Meningococcal Vaccine. MMWR Morb Mortal Wkly Rep 2017; 66: 734-737.
55. Pittock SJ, Barnett M, Bennett JL, Berthele A, de Sèze J, Levy M, et al. Ravulizumab in Aquaporin-4-Positive Neuromyelitis Optica Spectrum Disorder. Ann Neurol 2023; 93(6): 1053-68.
56. Mao-Draayer Y, Thiel S, Mills EA, Chitnis T, Fabian M, Katz Sand I, et al. Neuromyelitis optica spectrum disorders and pregnancy: therapeutic considerations. Nat Rev Neurol 2020; 16(3): 154-70.
57. Vukusic S, Marignier R, Ciron J, Bourre B, Cohen M, Deschamps R, et al. Pregnancy and neuromyelitis optica spectrum disorders: 2022 recommendations from the French Multiple Sclerosis Society. Mult Scler 2023; 29(1): 37-51.
58. Chen B, Wu Q, Ke G, Bu B. Efficacy and safety of tacrolimus treatment for neuromyelitis optica spectrum disorder. Sci Rep 2017; 7(1): 831.
59. Miyamoto K, Kusunoki S. Intermittent plasmapheresis prevents recurrence in neuromyelitis optica. Ther Apher Dial 2009; 13(6): 505-8.

BOLESTI IZ SPEKTRA NEUROMIJELITISA OPTIKA (NMOSD): TERAPIJSKA RAZMATRANJA

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Sažetak

Bolesti iz spektra neuromijelitisa optika (NMOSD) su retka, ali potencijalno teška autoimuna oboljenja centralnog nervnog sistema (CNS) za koja je nedavno odobrena primena nekoliko bioloških terapija. Istorijski gledano, tretmani koji modifikuju NMOSD oslanjali su se na konvencionalne imunosupresive širokog spektra, kao što su azatioprin i mofetil mikofenolat. Od 2015. godine, akumulirani su dokazi koji, s jedne strane, podržavaju biološku terapiju koja nije dokazano-efektivna (rituksimab) u okviru kontrolisane studije, a sa druge su omogućili odobravanje satralizumaba, inebilizumaba, ekulizumaba i ravulizumaba, posle sprovedenih kontrolisanih, randomizovanih klinikih studija kojima je dokazana njihova efikasnost i bezbednost. Ova sledeća generacija lekova pruža nekoliko ciljanih opcija lečenja za NMOSD koje modifikuju bolest. Ovde prvo prikazujemo mehanizam dejstva povezan sa njihovim specifičnim ciljevima. Zatim prikazujemo ključne dokaze koji podržavaju njihovu upotrebu u praksi. Trenutne terapijske opcije u NMOSD obuhvataju tri ciljana mehanizma u različitim fazama jedinstvene kaskade oštećenja tkiva CNS: uništavanje B-ćelija, anti-citokinske i terapije protiv komplementa. Po jedan lek iz svake klase odobren je na tržištu. Trenutni konsenzus predlaže pozicioniranje odobrenih lekova kao tretmana prve linije za novodijagnostikovane pacijente i kao alternativne terapije u slučaju neuspeha prethodnog lečenja.

Ključne reči: bolesti iz spektra neuromijelitisa optika, tretman, lekovi koji menjaju prirodni tok bolesti

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REVIEW ARTICLE

Life of the cell: is it important how cells die?

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Summary

Cell death emerges during embryonic development, and is preserved after the birth as an important process for maintaining homeostasis by removing damaged or aged cells. Two forms of cell deaths exist: accidental and regulated cell death. Necrosis is an accidental, unregulated, passive form of cell death that occurs due to the collapse of cellular homeostatic mechanisms under extreme non-physiological conditions. Regulated cell death is an active, energy-dependent process that functions as a physiological mechanism for maintaining homeostasis and in numerous pathological conditions when it provides selective elimination of potentially dangerous or infected cells. There are many types of regulated cell death: intrinsic and extrinsic types of apoptosis, autophagy dependent cell death, necroptosis, pyroptosis, ferroptosis, parthanatos, mitochondrial permeability transition-driven necrosis, lysosome-dependent cell death, immunogenic cell death, entosis and NET-osis. Different types of cell death are interconnected. Abnormal activation of the different forms of cell death can cause diseases. Dysregulation of the apoptotic program can lead to hyperplasia, autoimmune diseases and tumorigenesis, pyroptosis is associated with bacterial infection and necroptosis with human inflammatory skin diseases and carcinogenesis. Understanding the regulatory mechanisms of apoptosis led to the discovery of BH3 mimetics, drugs used for treatment of some types of B cell malignancies. Drugs that target necroptosis, pyroptosis and autophagy are under investigation and could be potentially used in future as therapies for various diseases, including cancer. The aim of this review is to summarize new knowledge about the processes of cell death, and to emphasize the importance of newly discovered molecular pathways regulating various types of cell death, enhancing our comprehension of health and disease.

Key words: cell death, necrosis, apoptosis, autophagy

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INTRODUCTION

The life of a cell, like the life of an organism, ultimately ends in death (1). Cell death emerges during embryonic development, playing a crucial role in morphogenesis and is preserved postnatally as an important process for maintaining homeostasis by removing damaged or aged cells (1). Cell death can occur as a component of physiological processes at the end of the cell's lifecycle, or due to the action of pathological factors that irreversibly damage cells (2). Therefore, there are two forms of cell deaths: regulated cell death and accidental cell death (1).

Accidental cell death was initially observed by Karl Vogt in 1842, but the concept of cell death and the terms necrosis and necrobiosis were introduced for the first time by Rudolf Virchow, in 1858 (3). Regulated cell death, or apoptosis, was morphologically described in 1885 by Walter Fleming and originally named chromatolysis, while the concept of programmed cell death was introduced later in 1950s, and named apoptosis by Kerr, Willy and Curie, in 1972 (3,5,6). Research on cell death started at the Institute of Histology and Embryology, Faculty of Medicine in Belgrade when its founder Aleksandar Dj. Kostić described cells with morphological characteristics of apoptosis in his doctoral dissertation, in 1921, and continued in 1980s (4). In 1990s, it was hypothesized that autophagy, the process of degradation of cellular components inside lysosomes, first observed in 1960s by electron microscopy, can also lead to cell death and Klionsky and Yoshinori began detailed research into the mechanisms of this process which later led to Nobel Prize-winning discoveries (7, 8,9).

In the beginning of the 21-century, cell death was classified according to its morphological characteristics into apoptosis, autophagy, necrosis and mitotic catastrophe (10), while in 2018, the current classification of cell death into accidental and regulated cell death (RCD) was postulated. Necrosis is an accidental, unregulated, passive form of cell death that occurs due to the collapse of cellular homeostatic mechanisms under extreme non-physiological conditions (2). RCD, or programmed cell death is an active, energy-dependent process (11). It occurs as a physiological process, during the development and maintenance of homeostasis and in numerous pathological conditions when it provides for selective elimination of potentially dangerous or infected cells (2). There are many types of regulated cell death, like apoptosis, necroptosis, pyroptosis, cell death dependent on autophagy, etc. **(Figure 1)** (2). Morphologically, all forms of cell death still may exhibit different combinations of microscopic features of apoptosis, autophagic cell death and necrosis (2). Even though various types of cell death

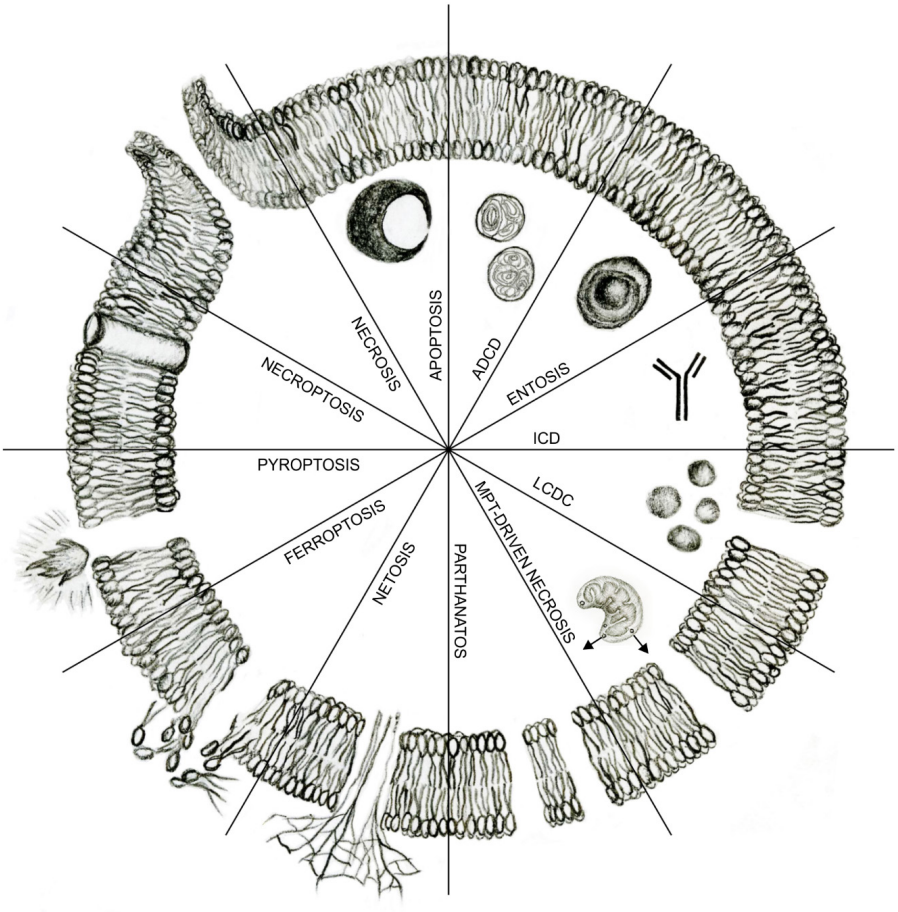

Figure 1. Classification of cell death. Types of cell death: apoptosis, ADCD (autophagy-dependent cell death), entosis, ICD (immunogenic cell death), LCDC (lysosome dependent cell death), MPT (mitochondrial permeability transition)-driven necrosis, parthanatos, NETosis, ferroptosis, pyroptosis, necroptosis, necrosis

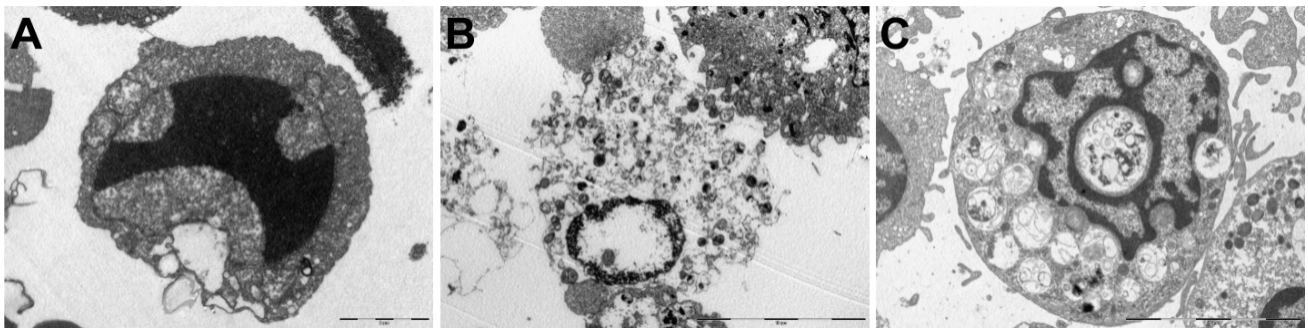

Figure 2. Transmission electron microscopy images of different types of cell death: lymphocyte apoptosis (A), necrosis of the B16 cell (B), autophagy in lymphocytes (C). Magnification 8900x (A, C) and 3500x (B)

have been discovered, including recently described cuproptosis and paraptosis, in this review we discussed only the types of cells death included in the latest classification of cell death (2).

The aim of this review is to summarize new knowledge about the processes of cell death, and to emphasize the importance of newly discovered molecular pathways regulating various types of cell death, enhancing our comprehension of health and disease.

NECROSIS

Necrosis is an unregulated form of cell death induced by external injury, independent of any signaling pathways or cellular energy expenditure, and is morphologically characterized by edema (swelling) of membrane organelles as well as swelling of the entire cell (oncosis) (11). The morphological hallmark of necrosis is the disruption of cell membrane integrity accompanied by the leakage of cellular contents into the extracellular space that always triggers an inflammatory response and local damage to neighboring cells **(Figure 2b)** (11).

Necrosis that occurs after apoptosis or autophagic cell death, when ATP is depleted, is called secondary necrosis (11). In addition to energy-independent passive necrosis, there are also regulated forms of necrosis that do require energy (11).

APOPTOSIS

Apoptosis or "cellular suicide" is a genetically regulated process, in which cell undergoes a characteristic sequence of morphological changes, including condensation of chromatin, typically resembling a crescent moon, organelle compaction, cytoplasmic condensation, cell shrinkage, and finally, fragmentation of the cell into apoptotic bodies by cell blebbing (6). The morphological characteristics of apoptosis, including chromatin and cytoplasmic condensation while the organelles remain intact, can be observed with transmission electron microscopy that is still considered to be golden standard for apoptosis identification **(Figure 2a)** (7). During apoptosis, membranes remain intact, preventing the release of cellular contents into the extracellular environment; therefore, there is an absence of inflammatory response or tissue damage (1, 11). Phosphatidylserine is displayed on the cell surface of apoptotic cells and apoptotic bodies as an "eat me" signal for surrounding cells and macrophages that rapidly remove dying apoptotic cells and apoptotic bodies from extracellular space by the process of efferocytosis (1, 2, 11).

The key players in the process of apoptosis are the family of cysteine proteases, called caspases, which are found in healthy cells in the form of inactive zymogens with low-to-absent protease activity (1). Their cascade activation leads to the execution of the apoptotic program (1). Initiator caspases (caspase-2, -8, -9, -10) are normally monomeric with a long prodomain that serves as a docking site for assembly into a self-activating complex, built around homomeric interactions between death (DD), death effector (DED), and caspase activation and recruitment (CARD) domains. Downstream or executioner caspases (caspase-3, -6, -7) exist as preformed dimers that become activated when the cleavage of a connector between subunits forms an open active site. In the extrinsic pathway of apoptosis, signals for caspase activation come from surrounding cells or molecules that bind to membrane receptors, so-called death receptors (tumor necrosis factor receptor 1 (TNFR1), Fas/CD95, TNFRSF10A, and TNFRSF10B), leading to the activation of initiator caspases (Figure 3) (1). In the intrinsic pathway of apoptosis, signals for caspase activation originate from within the cell due to various damages of organelles such as nucleus, endoplasmic reticulum or Golgi apparatus (1). Although activation of apoptosis by intrinsic pathway in the damaged cell can be mediated both by cytosolic and mitochondrial pathways, mitochondria have a central place in this type of cell death (1). Mitochondrial outer membrane permeabilization (MOMP) leads to increased permeability to small molecules, including cytochrome c, and the formation of the apoptosome which is specialized for activating effector caspases (1). Active caspases target many proteins essential for cellular viability, hence triggering apoptotic cell death (1). Among more than 1500 identified caspase substrates there is endonuclease, an enzyme that breaks down DNA, leading to the characteristic condensation of chromatin in the nucleus (1).

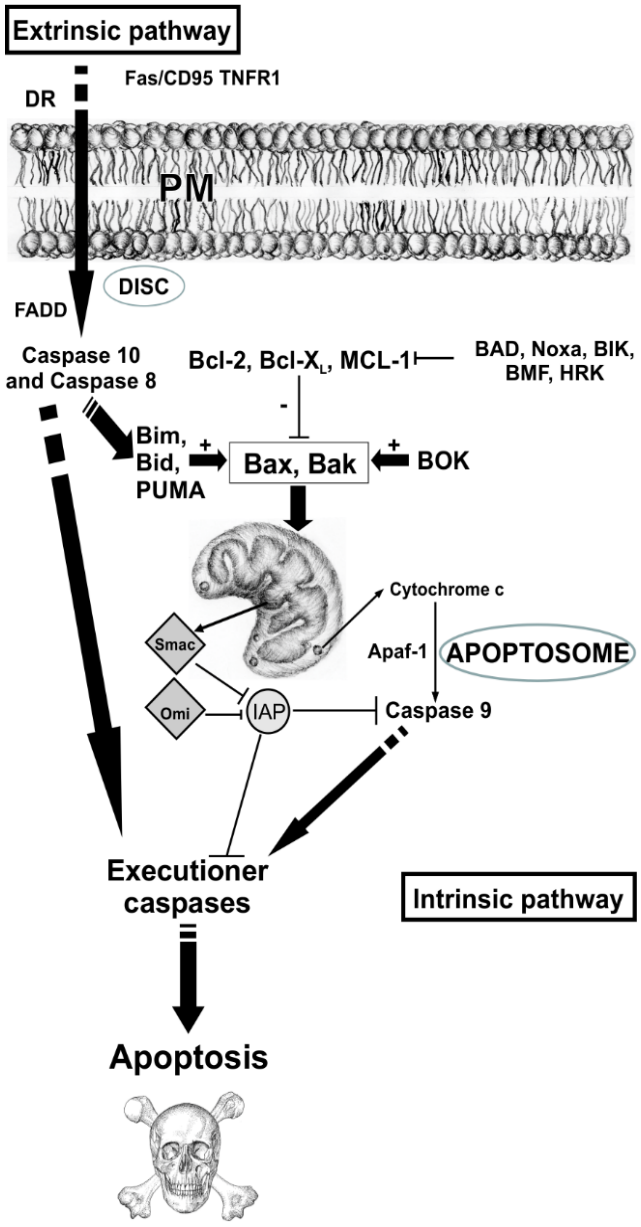

Figure 3. Extrinsic and intrinsic pathways of apoptosis. In extrinsic pathway ligand binding to death receptor Fas/CD95 makes conformational changes that help binding of its DD (death domain) with DD of FADD (Fas-associated death domain protein). A second domain in FADD, a DED, binds to DED domain in initiator caspases (caspase-8, and -10), leading to caspase dimerization. Caspase-activating assembly made of the death receptor, FADD, and caspase complex is called DISC (death-inducing signaling complex). In the intrinsic pathway, cytochrome c released from mitochondria binds to APAF-1 (apoptotic protease activating factor 1) and enables its oligomerization in heptameric wheel and exposure of its CARD domain. Interaction of CARD domain of APAF-1 with CARD in initiator caspase (caspase-9) helps docking of caspase-9 and is necessary for its proteolytic activity. This caspase-activating assembly platform, the apoptosome, is specialized for activating caspase-9 and -7, which have CARD-type prodomains. Interaction of Bcl-2 proteins (BAX, BAD, Bcl-2, BclXL, Mcl/1, BAD, Noxa, BIK, BMF, HRK, BOK, Bim, Bid, PUMA) regulate MOMP and cytochrome c release from the mitochondria. Cytochrome c release may be induced after Bid truncation by active caspase-8, linking the "extrinsic" and "intrinsic" pathways. Both pathways unite at the site of activation of executioner caspase-3 by upstream caspase-8, -9, or -10.

A number of regulatory proteins modulate the process of apoptosis. An important modulatory effect is exerted by proteins from the Bcl-2 (B-cell lymphoma-2) family of proteins **(Figure 3)** (1).

Various anti- and pro-apoptotic members of Bcl-2 family perform their actions at intracellular membranes (mitochondrial outer membrane, endoplasmic reticulum and nuclear membranes) and form a network of interactions that control MOMP (11). Their ability to selectively bind to each other is essential to their function in regulating MOMP and apoptosis (11). Bcl-2 family proteins selectively bind to each other via Bcl-2 homology domains (BH). The majority of proapoptotic and antiapoptotic Bcl-2 proteins are "multidomain" proteins that share sequence homology within 3–4 BH domains (12). A subset of proapoptotic Bcl-2 proteins show sequence homology with others only within the BH3 domain, death domain required for binding to "multidomain" Bcl-2 family members (12). These "BH3-only" molecules are: BID (BH3 interacting domain death agonist), BIM (Bcl-2 interacting mediator of cell death), BAD (Bcl-2 antagonist of cell death), Noxa, BIK (Bcl-2 interacting killer), BMF (Bcl-2 modifying factor), HRK (harakiri) and PUMA (p53 upregulated modulator of apoptosis) (12). Bcl-2 family interactions regulate mitochondrial intramembranous oligomerization of BAX (Bcl-2-associated X protein)/BAK (cl-2 antagonist killer 1), which is the key mechanism of MOMP (12). Antiapoptotic proteins, Bcl-2, BclXL (B-cell lymphoma extra-large) and Mcl-1 (myeloid cell leukemia sequence 1), inhibit apoptosis either by inhibiting BAX/BAK oligomerization or by engaging activator BH3-only proteins (12). "BH3-only" proteins activate apoptosis by both activating BAX/BAK oligomerization and by suppressing antiapoptotic proteins on the mitochondria and endoplasmic reticulum (12). BIM, BID and PUMA are "BH3-only" proteins known as "activators" that directly bind and trigger BAX/BAK oligomerization and bind and inhibit antiapoptotic Bcl-2 proteins (12). BAD, Noxa, BIK, BMF, HRK are BH3-only proteins known as "sensitizers" that bind and inhibit antiapoptotic Bcl-2 proteins (12). Disorders in the regulation of apoptosis are involved in the pathophysiology of a whole range of diseases (13, 14, 15). Overexpression of antiapoptotic molecules or downregulation of proapoptotic molecules was found in malignant cells resistant to apoptosis. High levels of Bcl-2 were first found in human follicular lymphomas and later in chronic lymphocytic leukemia cells (13). Abnormal expression of bcl2 family members, like Mcl-1 and BclXL is frequently found in many malignant tumors, like breast, gastric, prostate and hepatocellular carcinoma (14). Research data demonstrates that together with irregularities of pro-apoptotic BCL2 proteins and anti-apoptotic BCL2 proteins, aberrations of the components of the apoptosome and effector caspases also contribute to the pathogenesis of many cardiovas-

cular, hepatic, neurological, renal, autoimmune, inflammatory, infectious, and oncological diseases (14).

If a cell starts apoptosis and displays the nuclear morphology characteristic for apoptosis, but does not have enough energy to complete the initiated process of apoptosis, it may progress to secondary necrosis (16). Secondary necrosis is controlled by caspase-3 that cuts DFNA5 (deafness-associated tumor suppressor), into a necrosis-promoting DFNA5-N fragment that inserts into the plasma membrane, creating large pores that facilitate the release of inflammatory molecules into the extracellular space (16).

AUTOPHAGY-DEPENDENT CELL DEATH (ADCD)

Autophagy is an intracellular catabolic process responsible for the breakdown of damaged and/or non-functional cytoplasmic components and organelles, with the participation of lysosomal enzymes (17). Depending on the way material for degradation reaches a lysosomal lumen, three different types of autophagy are being described: chaperone mediated autophagy (CMA), microautophagy and macroautophagy (17).

In CMA, certain cytosolic proteins are first unfolded with a help of cytosolic chaperone proteins, after which they pass through a lysosomal membrane protein complex containing Lysosome-associated membrane protein 2A (LAMP2A) forming a distinct channel, thus reaching a lysosomal lumen where they are degraded (18).

In microautophagy, peculiar membrane invaginations of the lysosomal membrane are formed, projecting towards lysosomal lumen (19). After these invaginations are pinched off the lysosomal membrane, vacuoles that are formed, together with their cytosol-derived content, are degraded by lysosomal enzymes (19). In mammals, the process also takes place in endosomes (19), and in lysosomes it may include flap-like lysosomal membrane extensions, as sequestration mechanism (20).

Macroautophagy (hereafter referred to only as autophagy) relies on the formation of double membrane structures termed autophagosomes, which subsequently fuse with lysosomes (7). Autophagosomes are formed after a closure of cytoplasmic cisternal structures termed isolation membranes or phagophores, which sequester parts of the cytoplasm, including organelles, destined for degradation **(Figure 2c)** (7).

In mammalian cells, autophagy is regulated by two kinases: mTOR (Mammalian target of rapamycin) and AMPK (AMP-activated protein kinase) (Figure 4) (17, 21). In the presence of growth factors, autophagy is inhibited by mTOR which phosphorylates and inactivates another kinase ULK1 (Unc-51-like kinase 1) (17). On the other hand, AMPK acts as a cellular energy sensor **(Figure 4)** (22). When intracellular ATP/ADP ratio decreases, AMPK activates autophagy by phosphorylating and activating ULK1 and other proteins that regulate autophagy (23).

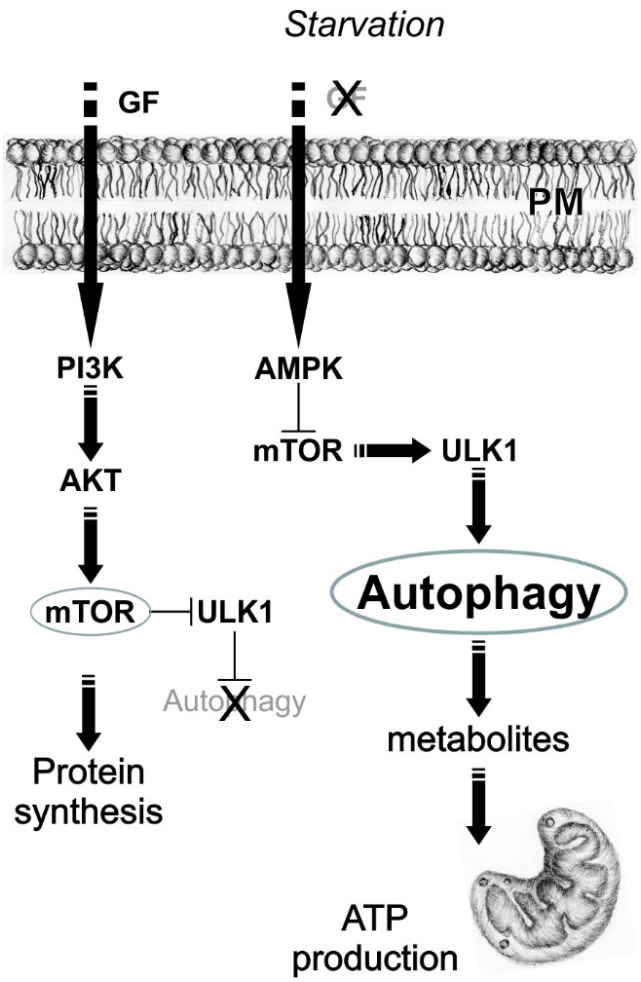

Figure 4. Activation of autophagy. Ligand binding to GF (growth factor) receptor leads to the activation of PI3K (phosphatidylinositol 3-kinase), AKT (protein kinase B) and mTOR that stimulates protein synthesis and inhibits autophagy through inactivation of ULK1. During nutrient starvation, AMPK, activates autophagy by inhibiting mTOR leading to ULK1 activation. Breakdown of organelles and proteins in autophagosomes produces metabolites that can be used for production of ATP in mitochondria.

Because it participates in the degradation of non-essential cytoplasmic components, recycling of their constituent molecules (e.g. during starvation) and removing of damaged and/or nonfunctional organelles and macromolecules, autophagy is generally considered to be cytoprotective (2). Blocking autophagy by artificial means generally leads to the acceleration of cellular destruction (rather than preventing cell death) (2, 24, 25). However, there are certain biological conditions where it is clear that excessive activation of autophagy may lead to cell death (2). Since the inhibition of autophagy in these circumstances rescues the cells, this type of cell death is being called autophagy-dependent cell death (2, 26).

This is different from previous cell death classifications (10), from the time when the cytoprotective role of autophagy was not properly acknowledged by the scientific community. Latest advances in the understanding of biological roles of autophagy enabled us to understand that a sheer presence of a large number of autophagy-related structures in dying cells is not enough to declare that

cells are dying by autophagy (2). Previously called autophagic cell death, it is now recognized that for the process of autophagy-dependent cell death to be demonstrated, it is not enough to notice that cells are dying "with" autophagy (27). Instead, it is necessary to prove that excessive autophagy is effectively killing the cells, by demonstrating that autophagy inhibition rescues them (2, 27).

In autophagy dependent cell death, cells actively participate, and the process is genetically regulated (2). This is a way some neurons die in rodent models of neonatal hypoxia-ischemia, and it also may occur in certain other pathological conditions (2, 28). In non-pathological conditions, autophagy-dependent cell death is also necessary as a mechanism of cell death in Drosophila metamorphosis (2).

OTHER FORMS OF CELL DEATHS

Necroptosis

Necroptosis is a type of RCD initiated by signals from the extracellular or intracellular microenvironment detected by death receptors (FAS, TNFR etc.) or pattern recognition receptors (PRRs) (2, 29). As the name suggests, necroptosis shares features with necrosis (early membrane disruption and cell and organelle swelling) and apoptosis (which is tightly regulated via genetics, signaling molecules, or toxins). In contrast to apoptosis, necroptosis is not only caspase-independent, but also induced by inhibition of caspase-8 (29, 30). The molecular markers of necroptosis are phosphorylated RIPK3 (receptor-interacting serine/threonine protein kinase 3) and phosphorylated MLKL (mixed lineage kinase domain like pseudokinase) **(Figure 5)** (1).

Necroptosis has been mostly investigated as the response to microbial infection. In cancer, necroptosis is beneficial for the antitumor immune response, not only because of MLKL-dependent cell lysis and uncontrolled release of cellular contents, but also because of tightly regulated activation of RIPK1/RIPK3/NFκB proinflammatory signaling, which leads to the synthesis of proinflammatory cytokines prior to cell disintegration (29).

As our understanding of the molecular mechanisms of necroptosis continues to evolve, it holds the potential to lead to innovative therapies and interventions in diverse fields of research, as it has been shown to play an important role not only in infections and systemic inflammatory response syndrome but in chronic pulmonary disease, acute kidney failure and fibrosis, liver disease, cardiovascular, neurodegenerative diseases and cancer (31).

Pyroptosis

Pyroptosis represents a unique form of RCD, primarily associated with the innate immune response to infections and inflammatory disorders. The term "pyroptosis"

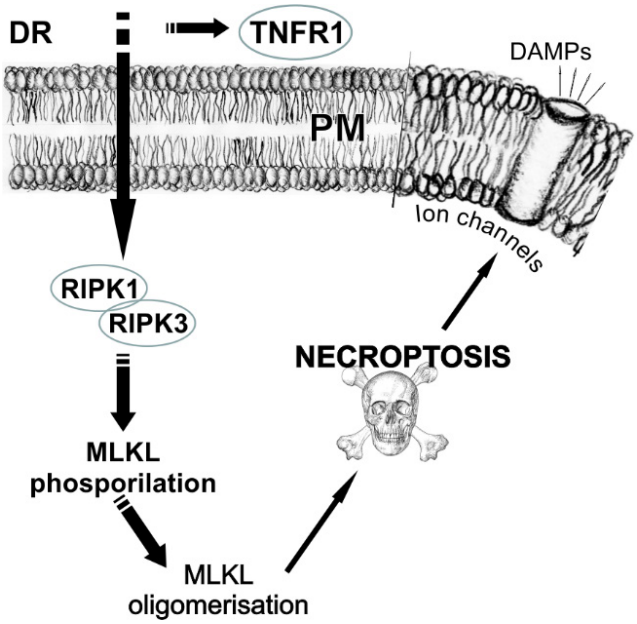

Figure 5. Molecular pathways of necroptosis. After ligation of TNFR-1, RIPK1 associates with RIPK3. RIPK3 phosphorylates the MLKL that oligomerizes after phosphorylation and promotes plasma membrane permeabilization. There are at least two pathways leading to the loss of cell integrity in necroptosis: MLKL could form a platform at the plasma membrane for the opening of calcium and sodium ion channels, enabling the influx of ions in the cell, cell swelling and rupture and/or MLKL itself could form pores in the plasma membranes.

was coined by D'Souza et al. in 2001, from Greek words pyro (fire or fever) and ptosis (falling), to emphasize the inflammatory nature of this type of cell death (32). For decades it was misconceived as a special form of apoptosis in monocytes, since it shared some features with apoptosis, like involvement of caspase-1 (33). Caspase-1, recognized as inflammatory caspase, is required for the cleavage of precursor pro-IL-1β into active IL-1β, also known as leukocytic pyrogen (34). Later, in 2002, the inflammasome was proposed to be a molecular platform for the activation of caspase-1 **(Figure 6)** (35).

Furthermore, it was demonstrated that pyroptosis could be induced in caspase-1 independent manner, by the activation of other caspases, specifically caspase-4, 5 and 11 (33). In 2015 it was discovered that both caspase-1 and caspase-4/5/11 share gasdermin D (GSDMD) as a key substrate in induction of pyroptosis, and since then, pyroptosis is commonly defined as gasdermin-mediated programmed cell death (33). The N-terminal domain of GSDMD can oligomerize to form pores in the cell membrane, causing cell swelling and lysis (33).

Although pyroptosis shares some features with apoptosis, like DNA fragmentation and intact nucleus, there are many differences between them, including the loss of membrane integrity, cellular swelling, the rupture of cell membrane and consequent inflammation (36).

The main role of pyroptosis, as an important player in the innate immunity, is defense against intracellular pathogens. However, numerous studies have pointed to a much

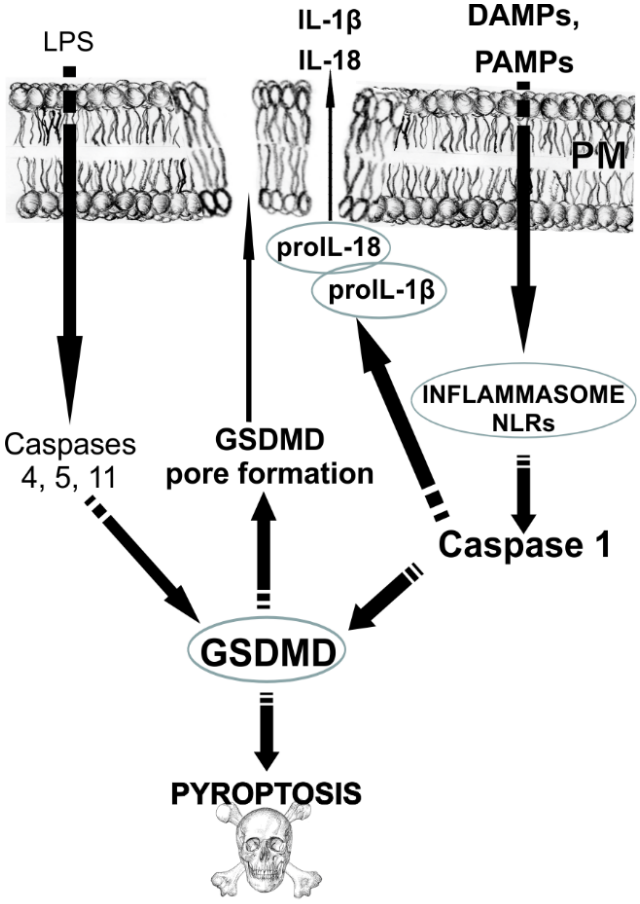

Figure 6. The mechanisms of pyroptosis. In canonical inflammatory pathways, PAMPs and DAMPs are detected by inflammasome, cytosolic multiprotein complex involving NLR (nucleotide-binding oligomerization domain (Nod)-like receptors) proteins which activate caspase-1. Caspase 1 performs cleavage of inflammatory cytokines IL-1β and IL-18 into mature forms, and cleavage of GSDMD that leads to formation of GSDMD pore and pyroptosis. In noncanonical inflammatory pathways, binding of LPS (lipopolysaccharides) leads to the cleavage of GSDMD, formation of GSDMD pore and pyroptosis.

broader aspect of pyroptosis as a type of RCD involved in inflammatory diseases and sepsis, cardiovascular, metabolic diseases, neurodegeneration and cancer (36).

Ferroptosis

Ferroptosis is a distinctive form of RCD, induced by iron-dependent, lipid peroxidation-mediated membrane damage. Although the term "ferroptosis" was coined not that long ago, in 2012, and ferroptosis-like cell death was described in 2001 as a cell death induced by oxidative stress ("oxytosis") (37), pioneering research was made back in the 1950s and 1960s, when the researchers observed cell death induced by cysteine-deprivation (38).

The primary system regulating ferroptosis is the cellular antioxidant system cysteine-glutathione (GSH)-glutathione peroxidase 4 (GPX4) (39). GPX4 is an antioxidant enzyme which catalyzes the reduction of lipid-hydrogen peroxides, cholesterol- and phospholipid hydrogen peroxides (PLOOHs), from cellular membranes and protects cells from the oxidative stress (40). Two compounds widely used for induction of ferroptosis, erastin and RSL3, act by interfering with GPX4-pathway that leads to the accumulation of PLOOHs in the cell and ferroptosis (39).

Potent inducers of ferroptosis include enzymes that directly oxygenate polyunsaturated fatty acids present in cellular membranes, such as lipoxygenases (LOXs), cytochrome P450 oxidoreductase (POR), and two membrane-remodeling enzymes, acyl-CoA synthetase long chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3). The importance of mitochondrial tricarboxylic acid cycle in lipogenesis, together with mitochondrial roles in beta-oxidation of fatty acids and oxidative metabolism, strongly links mitochondria with ferroptosis (39).

As the name suggests, iron has a central role in induction and regulation of ferroptosis. Iron is required as catalyst in numerous metabolic enzymes involved in reactive oxygen species (ROS) generation and lipid peroxidation, including LOXs and POR. Furthermore, intracellular iron ions can catalyze Fenton reaction, generating highly reactive hydroxyl radicals that initiate a chain reaction that culminates in lipid peroxidation and massive PLOOH production. Cellular processes that regulate iron homeostasis within cells (iron uptake, storage, utilization and efflux) therefore affect ferroptosis (41).

Cells undergoing ferroptosis exhibit swelling, with increased cell membrane density and membrane rupture. The distinctive feature of ferroptosis is the atrophy or condensation of mitochondria, disappearance of cristae and rupture of outer mitochondrial membrane (41).

A growing body of research indicates a potentially important role of ferroptosis in tumor suppression, ischemia-reperfusion injuries (also, associated with organ transplantation), immune surveillance, neurodegeneration and lung and liver fibrosis (39, 41).

Parthanatos

Parthanatos is a caspase-independent form of cell death which leads to DNA fragmentation (42). The term „parthanatos" is coined from „par", referring to poly(ADP)ribose (PAR), one of the key participants in this type of cell death, and „Thanatos", personification of death in Greek mythology. Parthanatos is a precisely regulated, multistep process resulting in large-scale DNA fragmentation and chromatin condensation (42).

One of the mechanisms for ensuring genome stability includes a nucleic enzyme called poly (ADP-ribose) polymerase 1 (PARP1). This DNA base-excision repair system facilitates DNA damage repair through the synthesis of PAR polymer (43). However, in instances of excessive DNA damage, such as ROS, inflammation, ischemia, hypoxia, etc., PARP1 becomes hyperactivated. Hyperactivation of PARP1 is the initial step in parthanatos, resulting in the production of long-chained, branched PAR polymers (42).

PARP1 overactivation causes cellular energy depletion. Namely, PARP1 hyperactivation requires nicotinamide adenine dinucleotide (NAD^+) as a cofactor, which is an important cofactor in cellular metabolism, including ATP synthesis. On the other hand, resynthesis of NAD^+ requires many ATP molecules. Additionally, accumulation of PAR polymers causes translocation of apoptosis-inducing factor (AIF) from mitochondria to nucleus (44). AIF may be considered as a parthanatos "executor", leading to massive DNA fragmentation, chromatin condensation, membrane rupture and cell death.

Parthanatos as a form of cell death is found in many diseases, while PARP inhibitors are extensively explored for the pharmacological treatment of breast, ovarian, and colorectal cancer (45).

Mitochondrial permeability transition-driven necrosis

Mitochondrial permeability transition (MPT)-driven necrosis is a form of cell death caused by a sudden increase in the inner mitochondrial membrane (IMM) permeability to small molecules. This type of cell death is initiated by the increase in Ca^{2+} and ROS in the mitochondrial matrix (46). The crucial event in MPT-driven necrosis is the formation of mitochondrial permeability transition pore (mPTP) in the IMM. The formation of mPTP enables permeability of IMM for molecules up to 1.5 kDa in size. The sustained opening of the mPTP causes abrupt change in mitochondrial permeability leading to the loss of mitochondrial membrane potential, mitochondrial swelling, rupture of the outer mitochondrial membrane (OMM), disruption of cellular energy metabolism and finally, necrotic death. Opposite to sustained mPTP opening, transient mPTP opening is not associated with cell death. Such reversible mPTPs are permeable to small molecules up to 300 Da and play a role in the mitochondrial homeostasis of Ca^{2+} (47).

It was long considered that the main structural components of mPTP are voltage-dependent anion channel (VDAC) in the OMM, adenine nucleotide translocase (ANT) in the IMM, and cyclophilin D (Cyp-D) in the mitochondrial matrix. Numerous experiments confirmed that neither VDAC nor ANT are required for the induction of mPTP (48). However, Cyp-D was recognized as an important regulator of the mPTP opening. Cyp-D is not a structural pore component of mPTP; it is a Ca^{2+}-sensitive isomerase present in the mitochondrial matrix which translocate to the IMM and mediates mPTP opening.

MPT-driven necrosis has been implicated in the pathogenesis of ischemic heart and brain disease, and many degenerative diseases which is why targeting its molecular steps might translate into novel therapeutic approaches (49).

Lysosome-dependent cell death

Lysosome-dependent cell death (LDCD) represents a form of RCD which is characterized by primary lysosomal membrane permeabilization (LMP), a phenomenon that leads to cell death (2). LMP is characterized by the release of lysosomal contents, including proteolytic enzymes of the cathepsin family, into the cytosol where they act in various ways as executors of cell death (50). LMP may occur downstream of MOMP and represent an epiphenomenon of intrinsic apoptosis (51). Alternatively, lysosomes can be permeabilized prior to mitochondria, which may involve the recruitment of BAX to the lysosomal membrane and formation of pores (52). Additional triggers of LPM may include lysosomotropic agents (e.g., sphingosine), calpains, reactive oxygen species (ROS), STAT3 etc. (53).

Cathepsins can catalyze proteolytic activation or inactivation of BID, BAX, anti-apoptotic BCL2 family members and XIAP, and therefore lead to LCDC with the involvement of MOMP and caspases (54). However, MOMP and caspases are not necessarily involved in LDCD so this type of RCD does not always exhibit apoptotic morphology (55).

LDCD is involved in different pathological and physiological conditions, such as intracellular pathogen response, inflammation, neurodegeneration, cardiovascular disorders, aging and tissue remodeling during involution of mammary gland after lactation (2, 50).

Immunogenic cell death

Immunogenic cell death (ICD) represents a form of RCD that is capable of initiating adaptive immune response in an immunocompetent host (2). This adaptive immune response is specific for endogenous (cellular) or exogenous (viral) antigens that are expressed by dying cells (2).

Various stimuli can initiate ICD, including viral infection, specific forms of radiation therapy, some FDA-approved chemotherapeutics and hypericin-based photodynamic therapy (2). These agents initiate release of damage-associated molecular patterns (DAMPs), such as calreticulin, ATP, type I IFN, cancer cell-derived nucleic acids, high-mobility group box 1 (HMGB1) and annexin A1 (ANXA1) by dying cells. DAMPs are being recognized by PRRs on immune system cells leading to the activation of an immune response with the formation of immunological memory (2, 56). Calreticulin relocates from the endoplasmic reticulum to the outer leaflet of plasma membrane where it functions as an "eat me" signal for DCs, macrophages and neutrophils and acts as a trigger for Th17 cell priming (57). ATP has a role as a "find-me" signal for dendritic cell precursors and macrophages and activates inflammasome (58). Cancer cells which are going to die by ICD release nucleic acids which can be taken up by DCs, macrophages and neutrophils and this results with the activation of type I IFN immune response (59).

Entosis

Entotic cell death, also known as entosis or cellular cannibalism, is a type of cell death in which a cell invades a living neighboring cell and eventually dies after being engulfed. This is a characteristic type of non-apoptotic cell death, which is recognizable by its cell-in-cell phenomenon, and which occurs in human tumors and non-tumor tissues, such as epithelial cell cultures (60). Entosis has several interesting features that distinguish it from other types of cell death, mainly that the entrapped cell can survive and even divide in the host cell, or it can leave the invaded cell without any sign of degradation (61). Although present in both physiological and pathological conditions, the exact role of entosis remains unclear with literature data suggesting both pro- and anti-tumorigenic effects. It is believed that entosis allows cancers to be removed by their healthy neighbors, as the tumorigenic cells have lost their cell-cell connections, and on the other hand, allows surrounding cells to survive by promoting cell competition (62). Entosis has also been associated with the process of embryo implantation (63), the elimination of spermatozoa by the Sertoli cells (64), but also in the pathophysiology of non-cancerous conditions such as diabetic cardiomyopathy (65). Several studies have shown that different drugs can induce entosis in various cancer cell lines. Methylselenoesters, novel synthesized selenium compounds, have caused entosis by cell detachment in pancreatic cancer cells (66). Recently published papers have shown that known and well-studied cytotoxic drugs, such as nintedanib and doxorubicin in combination with calcifediol, can induce entosis in prostate and breast cancer cells (67). These data highlight the possible therapeutic implications of entosis in cancer management.

NETosis

One of the mechanisms through which neutrophils destroy microbes is the formation of Neutrophil Extracellular Traps (NETs), which represent web-like structures made from modified chromatin and antimicrobial proteins, both originating from granules and nucleus of neutrophilic granulocytes (68). These formations bind, entrap, and finally destroy microorganisms in the extracellular space, without the need for intracellular phagocytosis. However, releasing these neutrophilic molecules/enzymes and pathogen destruction elicits neighboring tissue destruction and inflammation (69). During this process, neutrophils die, which is why this atypical type of cell death is termed NETosis. Unlike apoptosis, NETosis is characterized by the disintegration of nuclear and cytoplasmic membranes, followed by the leaking of genetic material in the extracellular space which immobilizes microorganisms (70). Though NETosis is a defensive reaction of the body, the release of intracellular granule components in the extracellular space causes proinflammatory reactions that can exacerbate existing inflammation in patients with different forms of autoimmune diseases (71). The role of NETosis in cancer progressions and metastasis is now also known, as NETosis can cause a wide range of changes needed for further development and dissemination of cancerous cells (72). NETosis can induce epithelial-mesenchymal transition in different cancers, create an optimal microenvironment for tumor development (73), and play a role in different cancer-related complications, such as venous thromboembolism (74).

CONCLUSION

Why is it important how a cell dies? In some types of cell death damaged cells do not have a preserved cell membrane and release DAMPs, therefore they are pro-inflammatory and lead to the activation of macrophages and dendritic cells (11). In contrast, apoptosis is an immunologically silent cell death, during which there is no spillage of cell contents into the environment due to the preserved cell membrane and the formation of apoptotic bodies, as well as due to the activation of caspases that inactivate DAMPs (11). Necrosis and pyroptosis are proinflammatory cell deaths during which proinflammatory cytokines are secreted, cell membrane bursts and cellular contents spill into the extracellular space leading to the damage of surrounding cells (11).

It may seem that apoptosis is a favorable form of cell death and that any pro-inflammatory form of cell death is always unfavorable and probably a result of an error in the activation of apoptosis. Nevertheless, pro-inflammatory forms of cell death may have evolved in order to remove multiple cells at the same time. Cells dying from pyroptosis, prevent the spread of infection by killing groups of cells that release DAMPs and recruit immune cells to the site of infection (75). Damaged or malignant cells dying from necroptosis attract immune cells that result in the death of malignant cell population and prevention of metastases (11). Therefore, the presence of regulated, proinflammatory cell death is in some circumstances important for the protection of surrounding cells and the organism.

Different types of cell death are interconnected. Apoptosis may inhibit the process of autophagy by caspase cleavage of Beclin and ATG (1). Furthermore, autophagy can inhibit apoptosis by increasing BCL-XL expression (1). The inactivation of autophagy during starvation leads to apoptosis, unless apoptosis is inactivated (e.g., BAX/BAK deficiency), in which case cell death occurs through necrosis (1). Inhibition of autophagy can either lead to mitochondrial dysfunction and apoptosis or to stabilization of RIPK1 and necroptosis (24, 25).

Abnormal activation of the different forms of cell death can cause diseases. Dysregulation of apoptotic pro-

gram can lead to hyperplasia, autoimmune diseases and tumorigenesis (76). Pyroptosis has a crucial place in the initiation of inflammatory response and achieving elimination of intracellular microorganism during bacterial infection, but uncontrolled pyroptosis can lead to organ failure and sepsis (77). Necroptosis has been associated with carcinogenesis related to non-alcoholic fatty liver disease and with human inflammatory skin diseases (78).

Understanding how cells dye and the knowledge about regulation mechanisms of different types of cell death, especially apoptosis, resulted in discovery of drugs that act as BH3 mimetics, ABT-737 and its oral derivative, Navitoclax, for the treatment of B cell malignancies that overexpress anti-apoptotic BCL-2 proteins (79). Drugs that target necroptosis, pyroptosis and autophagy are currently being researched and may serve as future therapeutic options for various diseases, including cancer.

Conflicts of interest

None to declare.

Author contributions

VB, TKS, MCM, TM made the design of the manuscript, review and editing of the text. The following sections of the manuscript were written by: TKS – abstracts, introduction and conclusion, TM – necrosis and apoptosis, DC – ACDC, SD – necroptosis, pyroptosis and ferroptosis, JR – parthanatos and (MPT)-driven necrosis, IP – LCDC and ICD, IZ – entosis and NETosis; MCM designed and made all figures. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Newton K, Strasser A, Kayagaki N, Dixit VM. Cell death. Cell. 2024 Jan 18;187(2):235-256. doi: 10.1016/j.cell.2023.11.044. PMID: 38242081.
2. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P et al. Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell Death Differ. 2018 Mar;25(3):486-541. doi: 10.1038/s41418-017-0012-4. PMID: 29362479; PMCID: PMC5864239.
3. Park W, Wei S, Kim BS, Kim B, Bae SJ, Chae YC, et al. Diversity and complexity of cell death: a historical review. Exp Mol Med. 2023 Aug;55(8):1573-1594. doi: 10.1038/s12276-023-01078-x. Epub 2023 Aug 23. Erratum in: Exp Mol Med. 2023 Sep;55(9):2083. doi: 10.1038/s12276-023-01107-9. PMID: 37612413; PMCID: PMC10474147.
4. Kopeina GS, Zhivotovsky B. Programmed cell death: Past, present and future. Biochem Biophys Res Commun. 2022 Dec 10; 633:55-58. doi: 10.1016/j.bbrc.2022.09.022. PMID: 36344162.
5. **Kravic-Stevovic T**, Bajcetic M, Mircic A. 100 godina Instituta za histologiju i embriologiju Aleksandar Dj. Kostic. Beograd: Medicinski fakultet Univerziteta; 2022. ISBN: 978-86-7117-654-5
6. Lockshin RA. One-half century (or more) of study of cell death: origins, present, and perhaps future. Frontiers in Cell Death.2023 June 16;2. https://www.frontiersin.org/journals/cell-death/articles/10.3389/fceld.2023.1197400. doi:10.3389/fceld.2023.1197400. ISSN:2813-5563
7. Klionsky DJ,, Bumbasirevic V, **Kravic-Stevovic T** et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016;12(1):1-222. doi: 10.1080/15548627.2016.1147886. PMID: 26799652
8. Kirkin V. History of the Selective Autophagy Research: How Did It Begin and Where Does It Stand Today? J Mol Biol. 2020 Jan 3;432(1):3-27. doi: 10.1016/j.jmb.2019.05.010. Epub 2019 May 11. PMID: 31082435; PMCID: PMC6971693.
9. Proikas-Cezanne T, Thumm M. Autophagy-from yeast to humans: Thirty years of molecular autophagy. FEBS Lett. 2024 Jan;598(1):3-6. doi: 10.1002/1873-3468.14796. PMID: 38206618.
10. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P, et al. Nomenclature Committee on Cell Death. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ. 2005 Nov;12 Suppl 2:1463-7. doi: 10.1038/sj.cdd.4401724. PMID: 16247491.
11. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biology International. 2019: 43: 582–592. doi: 10.1002/cbin.11137. Epub 2019 Apr 25. PMID: 30958602.
12. Czabotar PE, Garcia-Saez AJ. Mechanisms of BCL-2 family proteins in mitochondrial apoptosis. Nat Rev Mol Cell Biol. 2023 Oct;24(10):732-748. doi: 10.1038/s41580-023-00629-4. Epub 2023 Jul 12. PMID: 37438560.
13. Kravić-Stevović T, Bogdanović A, Bumbasirević V. Higher percentage of in vitro apoptotic cells at time of diagnosis in patients with chronic lymphocytic leukemia indicate earlier treatment requirement: ten years follow up. Srp Arh Celok Lek. 2014 Jan-Feb;142(1-2):48-53. doi: 10.2298/sarh1402048k. PMID: 24684031
14. Vitale I, Pietrocola F, Guilbaud E, Aaronson SA, Abrams JM, Adam D, et al. Apoptotic cell death in disease-Current understanding of the NCCD 2023. Cell Death Differ. 2023 May;30(5):1097-1154. doi: 10.1038/s41418-023-01153-w. PMID: 37100955; PMCID: PMC10130819.
15. Pljesa-Ercegovac M, Savic-Radojevic A, **Kravic-Stevovic T,** Bumbasirevic V, Mimic-Oka J, Simic T. Co-localization of GSTP1 and JNK in transitional cell carcinoma of urinary bladder. Genet Mol Biol. 2010;33(3):460-2. doi: 10.1590/s1415-47572010005000063. Epub 2010 Sep 1. PMID: 21637416.
16. Mázló A, Tang Y, Jenei V, Brauman J, Yousef H, Bácsi A, et al. Resolution Potential of Necrotic Cell Death Pathways. Int J Mol Sci. 2022 Dec 20;24(1):16. doi: 10.3390/ijms24010016. PMID: 36613458; PMCID: PMC9819908.
17. Yamamoto H, Matsui T. Molecular Mechanisms of Macroautophagy, Microautophagy, and Chaperone-Mediated Autophagy. J Nippon Med Sch. 2024 Mar 9;91(1):2-9. doi: 10.1272/jnms.JNMS.2024_91-102. Epub 2023 Jun 2. PMID: 37271546.
18. Valdor R, Martinez-Vicente M. The Role of Chaperone-Mediated Autophagy in Tissue Homeostasis and Disease Pathogenesis. Biomedicines. 2024 Jan 23;12(2):257. doi: 10.3390/biomedicines12020257. PMID: 38397859; PMCID: PMC10887052.
19. Krause GJ, Kirchner P, Stiller B, Morozova K, Diaz A, Chen KH, et al. Molecular determinants of the crosstalk between endosomal microautophagy and chaperone-mediated autophagy. Cell Rep. 2023 Dec 26;42(12):113529. doi: 10.1016/j.celrep.2023.113529. Epub 2023 Dec 6. PMID: 38060380; PMCID: PMC10807933.
20. Kuchitsu Y, Taguchi T. Lysosomal microautophagy: an emerging dimension in mammalian autophagy. Trends Cell Biol. 2024 Jul;34(7):606-616. doi: 10.1016/j.tcb.2023.11.005. Epub 2023 Dec 15. PMID: 38104013.
21. Vucicevic L, Misirkic M, Janjetovic K, Vilimanovich U, Sudar E, Isenovic E, Prica M, Harhaji-Trajkovic L, **Kravic-Stevovic T**, Bumbasirevic V, Trajkovic V. Compound C induces protective autophagy in cancer cells through AMPK inhibition-independent blockade of

Akt/mTOR pathway. Autophagy. 2011 Jan;7(1):40-50. doi: 10.4161/auto.7.1.13883. Epub 2011 Jan 1. PMID: 20980833.

22. Trefts E, Shaw RJ. AMPK: restoring metabolic homeostasis over space and time. Mol Cell. 2021 Sep 16;81(18):3677-3690. doi: 10.1016/j.molcel.2021.08.015. PMID: 34547233; PMCID: PMC8549486.
23. Lee Y, Tuan NM, Lee GJ, Kim B, Park JH, Lee CH. Regulatory Mechanisms Governing the Autophagy-Initiating VPS34 Complex and Its inhibitors. Biomol Ther (Seoul). 2024 Nov 1;32(6):723-735. doi: 10.4062/biomolther.2024.094. Epub 2024 Oct 7. PMID: 39370737; PMCID: PMC11535298.
24. Stamenkovic M, Janjetovic K, Paunovic V, Ciric D, **Kravic-Stevovic T,** Trajkovic V. Comparative analysis of cell death mechanisms induced by lysosomal autophagy inhibitors. Eur J Pharmacol. 2019 Sep 15;859:172540. doi: 10.1016/j.ejphar.2019.172540. Epub 2019 Jul 13. PMID: 31310755.
25. Isakovic AM, Dulovic M, Markovic I, **Kravic-Stevovic T,** Bumbasirevic V, Trajkovic V, Isakovic A. Autophagy suppression sensitizes glioma cells to IMP dehydrogenase inhibition-induced apoptotic death. Exp Cell Res. 2017 Jan 1;350(1):32-40. doi: 10.1016/j.yexcr.2016.11.001. Epub 2016 Nov 4. PMID: 27818246.
26. Ristic B, Bosnjak M, Arsikin K, Mircic A, Suzin-Zivkovic V, Bogdanovic A, Perovic V, Martinovic T, **Kravic-Stevovic T,** Bumbasirevic V, Trajkovic V, Harhaji-Trajkovic L. Idarubicin induces mTOR-dependent cytotoxic autophagy in leukemic cells. Exp Cell Res. 2014 Aug 1;326(1):90-102. doi: 10.1016/j.yexcr.2014.05.021. Epub 2014 Jun 5. PMID: 24907655.
27. Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, et al. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. Cell Death Differ. 2012 Jan;19(1):107-20. doi: 10.1038/cdd.2011.96. Epub 2011 Jul 15. PMID: 21760595; PMCID: PMC3252826.
28. Arsikin K, **Kravic-Stevovic T**, Jovanovic M, Ristic B, Tovilovic G, Zogovic N, et al. Autophagy-dependent and -independent involvement of AMP-activated protein kinase in 6-hydroxydopamine toxicity to SH-SY5Y neuroblastoma cells. Biochim Biophys Acta. 2012 Nov;1822(11):1826-36. doi: 10.1016/j.bbadis.2012.08.006. Epub 2012 Aug 16. PMID: 22917563.
29. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol. 2021 May;18(5):1106-1121. doi: 10.1038/s41423-020-00630-3. Epub 2021 Mar 30. PMID: 33785842; PMCID: PMC8008022.
30. Rodriguez DA, Quarato G, Liedmann S, Tummers B, Zhang T, Guy C, et al. Caspase-8 and FADD prevent spontaneous ZBP1 expression and necroptosis. Proc Natl Acad Sci U S A. 2022 Oct 11;119(41):e2207240119. doi: 10.1073/pnas.2207240119. Epub 2022 Oct 3. PMID: 36191211; PMCID: PMC9565532.
31. Choi ME, Price DR, Ryter SW, Choi AMK. Necroptosis: a crucial pathogenic mediator of human disease. JCI Insight. 2019 Aug 8;4(15):e128834. doi: 10.1172/jci.insight.128834. PMID: 31391333; PMCID: PMC6693822.
32. D'Souza CA, Heitman J. Dismantling the Cryptococcus coat. Trends Microbiol. 2001 Mar;9(3):112-3. doi: 10.1016/s0966-842x(00)01945-4. PMID: 11303499.
33. Shi J, Gao W, Shao F. Pyroptosis: Gasdermin-Mediated Programmed Necrotic Cell Death. Trends Biochem Sci. 2017 Apr;42(4):245-254. doi: 10.1016/j.tibs.2016.10.004. Epub 2016 Dec 5. PMID: 27932073.
34. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature. 1992 Apr 30;356(6372):768-74. doi: 10.1038/356768a0. PMID: 1574116.
35. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002 Aug;10(2):417-26. doi: 10.1016/s1097-2765(02)00599-3. PMID: 12191486.
36. Yu P, Zhang X, Liu N, Tang L, Peng C, Chen X. Pyroptosis: mechanisms and diseases. Signal Transduct Target Ther. 2021 Mar 29;6(1):128. doi: 10.1038/s41392-021-00507-5. PMID: 33776057; PMCID: PMC8005494.
37. Tan S, Schubert D, Maher P. Oxytosis: A novel form of programmed cell death. Curr Top Med Chem. 2001 Dec;1(6):497-506. doi: 10.2174/1568026013394741. PMID: 11895126.
38. Eagle H. Nutrition needs of mammalian cells in tissue culture. Science. 1955 Sep 16;122(3168):501-14. doi: 10.1126/science.122.3168.501. PMID: 13255879.
39. Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. Nat Rev Mol Cell Biol. 2021 Apr;22(4):266-282. doi: 10.1038/s41580-020-00324-8. Epub 2021 Jan 25. PMID: 33495651; PMCID: PMC8142022.
40. Ursini F, Maiorino M, Gregolin C. The selenoenzyme phospholipid hydroperoxide glutathione peroxidase. Biochim Biophys Acta. 1985 Mar 29;839(1):62-70. doi: 10.1016/0304-4165(85)90182-5. PMID: 3978121.
41. Chen X, Li J, Kang R, Klionsky DJ, Tang D. Ferroptosis: machinery and regulation. Autophagy. 2021 Sep;17(9):2054-2081. doi: 10.1080/15548627.2020.1810918. Epub 2020 Aug 26. PMID: 32804006; PMCID: PMC8496712.
42. Andrabi SA, Dawson TM, Dawson VL. Mitochondrial and nuclear cross talk in cell death: parthanatos. Ann N Y Acad Sci. 2008 Dec;1147:233-41. doi: 10.1196/annals.1427.014. PMID: 19076445; PMCID: PMC4454457.
43. Aredia F, Scovassi AI. Involvement of PARPs in cell death. Front Biosci (Elite Ed). 2014 Jun 1;6(2):308-17. doi: 10.2741/707. PMID: 24896207.
44. Daugas E, Nochy D, Ravagnan L, Loeffler M, Susin SA, Zamzami N, et al. Apoptosis-inducing factor (AIF): a ubiquitous mitochondrial oxidoreductase involved in apoptosis. FEBS Lett. 2000 Jul 7;476(3):118-23. doi: 10.1016/s0014-5793(00)01731-2. PMID: 10913597.
45. Rose M, Burgess JT, O'Byrne K, Richard DJ, Bolderson E. PARP Inhibitors: Clinical Relevance, Mechanisms of Action and Tumor Resistance. Front Cell Dev Biol. 2020 Sep 9; 8:564601. doi: 10.3389/fcell.2020.564601. PMID: 33015058; PMCID: PMC7509090.
46. Bonora M, Giorgi C, Pinton P. Molecular mechanisms and consequences of mitochondrial permeability transition. Nat Rev Mol Cell Biol. 2022 Apr;23(4):266-285. doi: 10.1038/s41580-021-00433-y. Epub 2021 Dec 8. PMID: 34880425.
47. Brenner C, Moulin M. Physiological roles of the permeability transition pore. Circ Res. 2012 Oct 12;111(9):1237-47. doi: 10.1161/CIRCRESAHA.112.265942. PMID: 23065346.
48. Basso E, Fante L, Fowlkes J, Petronilli V, Forte MA, Bernardi P. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. J Biol Chem. 2005 May 13;280(19):18558-61. doi: 10.1074/jbc.C500089200. Epub 2005 Mar 25. PMID: 15792954.
49. Bonora M, Patergnani S, Ramaccini D, Morciano G, Pedriali G, Kahsay AE, et al. Physiopathology of the Permeability Transition Pore: Molecular Mechanisms in Human Pathology. Biomolecules. 2020 Jul 4;10(7):998. doi: 10.3390/biom10070998. PMID: 32635556; PMCID: PMC7408088.
50. Berg AL, Rowson-Hodel A, Wheeler MR, et al. Engaging the Lysosome and Lysosome-Dependent Cell Death in Cancer. In: Mayrovitz HN, editor. Breast Cancer [Internet]. Brisbane (AU): Exon Publications; 2022 Aug 6. Chapter 13. Available from: https://www.ncbi.nlm.nih.gov/books/NBK583821/ doi: 10.36255/exon-publications-breast-cancer-lysosome
51. Huai J, Vögtle FN, Jöckel L, Li Y, Kiefer T, Ricci JE, et al. TNFα-induced lysosomal membrane permeability is downstream of MOMP and triggered by caspase-mediated NDUFS1 cleavage and ROS formation. J Cell Sci. 2013 Sep 1;126(Pt 17):4015-25. doi: 10.1242/jcs.129999. Epub 2013 Jun 20. PMID: 23788428.
52. Feldstein AE, Werneburg NW, Li Z, Bronk SF, Gores GJ. Bax inhibition protects against free fatty acid-induced lysosomal permeabilization. Am J Physiol Gastrointest Liver Physiol. 2006 Jun;290(6):G1339-46. doi: 10.1152/ajpgi.00509.2005. Epub 2006 Feb 16. PMID: 16484678; PMCID: PMC3056273.
53. Gómez-Sintes R, Ledesma MD, Boya P. Lysosomal cell death mechanisms in aging. Ageing Res Rev. 2016 Dec; 32:150-168. doi: 10.1016/j.arr.2016.02.009. Epub 2016 Mar 3. PMID: 26947122.

54. Taniguchi M, Ogiso H, Takeuchi T, Kitatani K, Umehara H, Okazaki T. Lysosomal ceramide generated by acid sphingomyelinase triggers cytosolic cathepsin B-mediated degradation of X-linked inhibitor of apoptosis protein in natural killer/T lymphoma cell apoptosis. Cell Death Dis. 2015 Apr;6(4):e1717. doi: 10.1038/cddis.2015.82. PMID: 25855965 PMCID: PMC4650549

55. Et al. Distinct cathepsins control necrotic cell death mediated by pyroptosis inducers and lysosome-destabilizing agents. Cell Cycle. 2015;14(7):964-72. doi: 10.4161/15384101.2014.991194. PMID: 25830414; PMCID: PMC4614982.

56. Legrand AJ, Konstantinou M, Goode EF, Meier P. The Diversification of Cell Death and Immunity: Memento Mori. Mol Cell. 2019 Oct 17;76(2):232-242. doi: 10.1016/j.molcel.2019.09.006. Epub 2019 Oct 2. PMID: 31586546.

57. Aria H, Rezaei M. Immunogenic cell death inducer peptides: A new approach for cancer therapy, current status and future perspectives. Biomed Pharmacother. 2023 May; 161:114503. doi: 10.1016/j.biopha.2023.114503. Epub 2023 Mar 13. PMID: 36921539.

58. Fucikova J, Kepp O, Kasikova L, Petroni G, Yamazaki T, Liu P, et al. Detection of immunogenic cell death and its relevance for cancer therapy. Cell Death Dis. 2020 Nov 26;11(11):1013. doi: 10.1038/s41419-020-03221-2. PMID: 33243969; PMCID: PMC7691519.

59. Rodrigues MC, Morais JAV, Ganassin R, Oliveira GRT, Costa FC, Morais AAC, et al. An Overview on Immunogenic Cell Death in Cancer Biology and Therapy. Pharmaceutics. 2022 Jul 27;14(8):1564. doi: 10.3390/pharmaceutics14081564. PMID: 36015189; PMCID: PMC9413301.

60. Florey O, Kim SE, Overholtzer M. Entosis: Cell-in-Cell Formation that Kills Through Entotic Cell Death. Curr Mol Med. 2015;15(9):861-6. doi: 10.2174/1566524015666151026100042. PMID: 26511711.

61. Kianfar M, Balcerak A, Chmielarczyk M, Tarnowski L, Grzybowska EA. Cell Death by Entosis: Triggers, Molecular Mechanisms and Clinical Significance. Int J Mol Sci. 2022 Apr 30;23(9):4985. doi: 10.3390/ijms23094985. PMID: 35563375; PMCID: PMC9102690.

62. Durgan J, Tseng YY, Hamann JC, Domart MC, Collinson L, Hall A, et al. Mitosis can drive cell cannibalism through entosis. Elife. 2017 Jul 11;6:e27134. doi: 10.7554/eLife.27134. PMID: 28693721; PMCID: PMC5505699.

63. Li Y, Sun X, Dey SK. Entosis allows timely elimination of the luminal epithelial barrier for embryo implantation. Cell Rep. 2015 Apr 21;11(3):358-65. doi: 10.1016/j.celrep.2015.03.035. Epub 2015 Apr 9. PMID: 25865893; PMCID: PMC5089169.

64. Ahmed N, Yang P, Huang Y, Chen H, Liu T, Wang L, et al. Entosis Acts as a Novel Way within Sertoli Cells to Eliminate Spermatozoa in Seminiferous Tubule. Frontiers in Physiology [Internet]. 2017 [cited 2023 Dec 7];8. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2017.00361

65. Chen Y, Hua Y, Li X, Arslan IM, Zhang W, Meng G. Distinct Types of Cell Death and the Implication in Diabetic Cardiomyopathy. Frontiers in Pharmacology [Internet]. 2020 [cited 2023 Dec 7];11. Available from: https://www.frontiersin.org/articles/10.3389/fphar.2020.00042

66. Khalkar P, Díaz-Argelich N, Antonio Palop J, Sanmartín C, Fernandes AP. Novel Methylselenoesters Induce Programed Cell Death via Entosis in Pancreatic Cancer Cells. Int J Mol Sci. 2018 Sep 20;19(10):2849. doi: 10.3390/ijms19102849. PMID: 30241340; PMCID: PMC6213452.

67. Abramczyk O, Stawieraj S, Mlicka A, Zielińska W, Niewiadomski P, Hałas-Wiśniewska M, et al. Effect of combined action of doxorubicin and calcifediol on MCF-7 breast cancer cells. Medical Research Journal. 2023;8(3):242–50.

68. Rada B. Neutrophil Extracellular Traps. Methods Mol Biol. 2019;1982:517-528. doi: 10.1007/978-1-4939-9424-3_31. PMID: 31172493; PMCID: PMC6874304.

69. DeLeo FR, Allen LH. Phagocytosis and neutrophil extracellular traps. Fac Rev. 2020 Dec 21; 9:25. doi: 10.12703/r/9-25. PMID: 33659957; PMCID: PMC7886055.

70. Pérez-Figueroa E, Álvarez-Carrasco P, Ortega E, Maldonado-Bernal C. Neutrophils: Many Ways to Die. Frontiers in Immunology [Internet]. 2021 [cited 2023 Dec 7];12. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2021.631821

71. Mutua V, Gershwin LJ. A Review of Neutrophil Extracellular Traps (NETs) in Disease: Potential Anti-NETs Therapeutics. Clin Rev Allergy Immunol. 2021 Oct;61(2):194-211. doi: 10.1007/s12016-020-08804-7. PMID: 32740860; PMCID: PMC7395212.

72. Ronchetti L, Boubaker NS, Barba M, Vici P, Gurtner A, Piaggio G. Neutrophil extracellular traps in cancer: not only catching microbes. J Exp Clin Cancer Res. 2021 Jul 14;40(1):231. doi: 10.1186/s13046-021-02036-z. PMID: 34261496; PMCID: PMC8281578.

73. Jaboury S, Wang K, O'Sullivan KM, Ooi JD, Ho GY. NETosis as an oncologic therapeutic target: a mini review. Frontiers in Immunology [Internet]. 2023 [cited 2023 Dec 8];14. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1170603

74. Snoderly HT, Boone BA, Bennewitz MF. Neutrophil extracellular traps in breast cancer and beyond: current perspectives on NET stimuli, thrombosis and metastasis, and clinical utility for diagnosis and treatment. Breast Cancer Res. 2019 Dec 18;21(1):145. doi: 10.1186/s13058-019-1237-6. PMID: 31852512; PMCID: PMC6921561..

75. Liu Y, Pan R, Ouyang Y, Gu W, Xiao T, Yang H, et al. Pyroptosis in health and disease: mechanisms, regulation and clinical perspective. Signal Transduct Target Ther. 2024 Sep 20;9(1):245. doi: 10.1038/s41392-024-01958-2. PMID: 39300122; PMCID: PMC11413206.

76. Kayagaki N, Webster JD, Newton K. Control of Cell Death in Health and Disease. Annu Rev Pathol. 2024 Jan 24; 19:157-180. doi: 10.1146/annurev-pathmechdis-051022-014433. Epub 2023 Oct 3. PMID: 37788577.

77. Brokatzky D, Mostowy S. Pyroptosis in host defence against bacterial infection. Dis Model Mech. 2022 Jul 1;15(7):dmm049414. doi: 10.1242/dmm.049414. Epub 2022 Jul 8. PMID: 35801644; PMCID: PMC10623139.

78. Morgan MJ, Kim YS. Roles of RIPK3 in necroptosis, cell signaling, and disease. Exp Mol Med. 2022 Oct;54(10):1695-1704. doi: 10.1038/s12276-022-00868-z. Epub 2022 Oct 12. PMID: 36224345; PMCID: PMC9636380.

79. Townsend PA, Kozhevnikova MV, Cexus ONF, Zamyatnin AA Jr, Soond SM. BH3-mimetics: recent developments in cancer therapy. J Exp Clin Cancer Res. 2021 Nov 9;40(1):355. doi: 10.1186/s13046-021-02157-5. PMID: 34753495; PMCID: PMC8576916.

ŽIVOT ĆELIJE: DA LI JE VAŽNO KAKO ĆELIJE UMIRU?

Tamara Kravić-Stevović[1], Tamara Martinović[1], Darko Ćirić[1], Jelena Rakočević[1], Ivana Paunković[1], Ivan Zaletel[1], Sanja Despotović[1], Mila Ćetković-Milisavljević[1], Vladimir Bumbaširević[1,2]

Sažetak

Ćelijska smrt je prisutna tokom embrionalnog razvoja, i posle rođenja kao važan proces, neophodan za održavanje homeostaze, kojim se uklanjaju ostarele i oštećene ćelije. Postoje dva tipa ćelijske smrti: akcidentalna i regulisana ćelijska smrt. Nekroza je akcidentalna, neregulisana, pasivna forma ćelijske smrti koja nastaje usled kolapsa homeostatskih mehanizama u ekstremnim nefiziološkim uslovima. Regulisana ćelijska smrt je aktivan, energetski zavistan proces, koji nastaje u fiziološkim uslovima, tokom održavanja homeostaze organizma i u brojnim patološkim stanjima kada obezbeđuje selektivnu eliminaciju potencijalno opasnih ili inficiranih ćelija. Brojni su tipovi regulisane ćelijske smrti: unutrašnji i spoljašnji tip apoptoze, ćelijska smrt zavisna od autofagije, nekroptoza, piroptoza, feroptoza, partanatos i MPT nekroza, ćelijska smrt zavisna od lizozoma, imunogena ćelijska smrt, entoza i NET-oza. Različiti tipovi ćelijske smrti su međusobno povezani. Abnormalna aktivnost različitih formi ćelijske smrti može dovesti do razvoja brojnih bolesti. Poremećaj regulacije apoptoze može dovesti do hiperplazije, razvoja autoimunskih oboljenja i tumora. Poznavanje regulacionih mehanizama apoptoze dovelo je do otkrića BH3 mimetika, lekova koji se koriste u terapiji nekih tipova malignih tumora B limfocita. U savremenim naučnim istraživanjima ispituju se lekovi koji utiču na nekroptozu, piroptozu i autofagiju koji mogu u budućnosti biti terapija za različite bolesti, uključujući i maligne tumore. Cilj ovog revijskog rada je da rezimira nova saznanja u vezi sa procesima ćelijske smrti i ukaže na značaj novootkrivenih molekularnih puteva regulacije različitih tipova ćelijske smrti u cilju boljeg razumevanja zdravlja i bolesti.

Ključne reči: ćelijska smrt, nekroza, apoptoza, autofagija

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REVIEW ARTICLE

Dilemmas in the differential diagnosis of pediatric multiple sclerosis

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Summary

Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disease of the central nervous system (CNS). It typically presents in early or middle adulthood, and pediatric-onset MS (POMS), defined by the first MS attack occurring before the age of 18, is less common. Current data from the Danish Multiple Sclerosis Registry indicates that nearly 3% of patients had the onset before the age of 18. In comparison with adult-onset MS, POMS patients typically have a more inflammatory-active disease course, resulting in more frequent relapses, but slower long-term disability accumulation. In POMS, diagnostic dilemmas may include differentiating MS from acute disseminated encephalomyelitis (ADEM) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), both of which most commonly occur among children and share some clinical and imaging features with MS. Inherited leukodystrophies should be considered in the differential diagnosis only in select cases, as their hallmark clinical feature—a progressive disease course—distinguishes them from the typically non-progressive acquired demyelinating syndromes, including neuromyelitis optica spectrum disorder. It's important to remember that magnetic resonance imaging (MRI) of the brain and spinal cord is essential for establishing a timely and accurate diagnosis of POMS. MRI helps exclude alternative diagnoses, allowing for the prompt initiation of effective treatment.

Keywords: multiple sclerosis, diagnosis, pediatric-onset

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INTRODUCTION

Multiple sclerosis (MS) is a chronic, neuroinflammatory and neurodegenerative disease of the central nervous system (CNS). MS typically presents in early or middle adulthood. Pediatric-onset MS (POMS), defined as the first MS attack occurring before the age of 18, is less common. Pediatric-onset multiple sclerosis (POMS) is estimated to account for approximately 2% to 10% of all MS cases, with disease onset before the age of 10 occurring in only 0.2% to 0.7% of cases (1). Current data from the Danish Multiple Sclerosis Registry, with onsets between 2003 and 2022, indicates that 2.89% of patients had the onset before the age of 18 (2). In the recent meta-analysis, an estimated global incidence of POMS was 0.87 per 100,000 individuals per year (3).

In the French KidbioSEP cohort, the mean age of onset was 11.8 ± 3.7 years. The study also suggested that the number of children experiencing their first attack before the age of 11 may be higher than previously estimated (4). Younger children present with encephalopathy, seizures, and multifocal deficits, more often than children over the age of 12 and adults. Moreover, in children under 12, T2-hyperintense lesions tend to be larger and confluent. Conversely, clinical presentations and imaging findings of older children are usually similar to those of adults (5). The clinical presentation of POMS is very heterogeneous and depends, first of all, on the location of the demyelinating plaques that affect predilection sites, that is, the optic nerve, brain and spinal cord (6). As already mentioned, in children with the first attack after the age of 11, the clinical picture of POMS does not differ significantly from adult MS. However, in younger children, symptoms of encephalopathy may occur more often, accompanied by a disturbance in the state of consciousness and epileptic seizures, followed by headache, nausea and vomiting (7). Studies to date indicate that the majority of POMS patients—approximately 50% to 70%—present with a multifocal, or polysymptomatic, disease onset, while a mono-focal presentation occurs in 30% to 50% of cases. However, the multifocal presentation most often occurs before the age of 12, while the mono-focal presentation of the disease occurs most often after the age of 10 (8).

In comparison with adult-onset MS, POMS patients typically have a more inflammatory-active disease course, resulting in more frequent relapses but slower long-term disability accumulation (9). These features are generally attributed to the extensive post-relapse recovery that can be at least partly attributed to a higher ability for myelin repair/synthesis and greater plasticity of the developing brain (10). Immunological changes that occur throughout the lifespan impact the clinical manifestations of MS, such as relapse frequency, severity, and recovery. Children have larger and higher proportions of naive T cells and higher B-cell functional capacities, resulting in more robust immune responses to antigens than adults, which may amplify the inflammatory pathology of MS and explain why most POMS cases present with a relapsing-remitting course (2). Approximately 98% of patients with POMS present with a relapsing-remitting disease course (11). Although progressive-onset multiple sclerosis is rare in childhood and the transition to a secondary progressive phenotype occurs over a longer period, patients with POMS still reach ambulatory disability milestones at younger chronological ages than those with adult-onset MS due to their earlier disease onset (12, 13).

Although POMS patients have relatively slower physical disability progression, the early and frequent neuroinflammatory attacks can result in impaired brain development and poorer cognitive performance when compared to adult-onset MS patients or non-MS peers (14, 15). These impairments can have long-term consequences, including a lower likelihood of pursuing higher education, lower annual earnings, frequent sick days during work life, and early enrollment into disability pension programs (16). Very recently, it has been emphasized that acquired demyelinating syndromes (ADS), such as MS and myelin oligodendrocyte glycoprotein antibody disease (MOGAD), often cause cognitive impairment and fatigue in children and adults (17). POMS is associated with worse cognitive impairment in adulthood compared to adult-onset MS and reduced participation in university education and employment. However, the impact of POMS on school participation remains unknown to date. Therefore, efforts toward early diagnosis, discovery of early predictors of long-term outcomes, and appropriate early drug intervention are highly warranted (18).

A group of neurological disorders characterized by acute or subacute onset of neurological deficits associated with the evidence of inflammatory demyelination of the central nervous system (CNS), including the optic nerves, is collectively named ADS (19), with acute disseminated encephalomyelitis (ADEM), occurring in 22-32% of children with ADS. It could be represented as one of neuroinflammatory diseases, such as AQP4-NMOSD, MOGAD, ADEM with encephalopathy, or a monophasic disease (20), or MS. Furthermore, it has to be emphasized that only 20% of pediatric ADS cases are ultimately diagnosed with POMS (21).

In POMS, diagnostic dilemmas may include differentiating MS from monophasic acute disseminated encephalomyelitis (ADEM) and myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) (20), both of which most commonly occur among children and share some clinical and imaging features with MS. Inherited leukodystrophies should be considered in the differential diagnosis only in select cases, as their hallmark feature—a progressive disease course—is uncommon among other acquired demyelinating syndromes (ADS) (22).

PATHOHISTOLOGY AND IMMUNOPATHOGENESIS IN MS

Demyelinated regions, i.e., lesions or plaques, in the gray and white matter of the CNS, form the pathological basis of MS. Nowadays, plaques of demyelination are known to involve both white and gray matter, including the nuclei, cortex, and spinal cord. Plaques indicate damage and loss of oligodendrocytes and myelin sheaths (23, 24). At the very beginning of the disease, both neurons and axons are partly preserved. However, later on, with the progression of the disease, there is neuroaxonal damage. Inflammation is most pronounced in the acute stages of the disease but can be detected in all stages. In addition to the demyelination process, remyelination can also be detected initially. In the initial stage of the disease, in the pathohistological findings, macrophages with CD8+ T lymphocytes are the most abundant, and plasma cells and B lymphocytes can also be found (23, 24).

Studies have shown that with the progression of the disease and neuroaxonal damage, there is a deterioration in the degree of disability and the occurrence of brain atrophy. In the pathohistological findings, diffuse fields of B and T lymphocytes, astrocytes, and microglia can be detected. In addition, damage to axons and myelin can also be seen. All these processes damage the white and gray matter of the brain and spinal cord and the consequent reduction in brain volume, i.e., atrophy (23, 24, 25). In the later course of the disease, sclerotic lesions are created in the white matter plaques under the influence of astrocytes. Postmortem analysis of MS brains, compared to those of healthy controls, showed a 19% to 24% reduction in the cross-sectional area of the spinal cord at the cervical, thoracic, and lumbar levels. Gray matter atrophy was from 17 to 21%, and white matter from 19% to 24% (24). The density of axons in patients was lower by 57% to 62% compared to healthy controls. Demyelination affected between 11% and 13% of white matter and 24% to 48% of gray matter (24).

It is crucial to emphasize that axon damage is the primary pathophysiological mechanism of disease progression and neurological disability. It is believed to occur very early in MS, even during radiologically isolated syndrome (RIS), and POMS patients have a greater degree of acute axonal damage than adults (24). This was also shown by pathohistological studies, which were conducted on autopsy tissues of nineteen children and adolescents with POMS and clinically isolated syndrome (CIS) (25). This study showed that acute axon damage is 50% higher in children and adolescents (age range from 4 to 17 years) than in adult patients (26, 27).

HOW TO ESTABLISH THE DIAGNOSIS OF POMS?

Although guidelines have evolved over the years, the critical role of magnetic resonance imaging (MRI) has remained consistently recognized. MRI is highlighted as the most valuable tool for diagnosing POMS. It has also been generally accepted that MRI is mandatory for demonstrating dissemination in time and space.

The diagnostic criteria proposed by Krupp et al., widely used since 2013, have evolved gradually since 2004, incorporating advancements—particularly in MRI technology—along the way (28). Krupp's diagnostic criteria for POMS are presented in **Table 1**.

Table 1. Diagnostic criteria for POMS by Krupp et al.

One of the following is necessary:
Two or more non-encephalopathic CNS events: Occurring at least30 days apart Affecting more than one area of the CNS
One non-encephalopathic CNS event and MRI features, according to 2010 Revised McDonald criteria for DIS and DIT
One ADEM episode followed three or more months later by: A non-encephalopathic clinical event New MRI lesions fulfilling 2010 Revised McDonald DIS criteria

CNS central nervous system; MRI magnetic resonance imaging; DIS dissemination in space; DIT dissemination in time; ADEM acute disseminated encephalomyelitis.
Adapted from Krupp et al. (28)

The current diagnostic criteria for adult MS patients (29) can be fully applied in children older than 12 years **(Table 2)** since the 2017 McDonald criteria are being increasingly validated in children who had not presented an ADEM as the first demyelinating event (4). However, in children below the age of 12, there is a diagnostic concern to using the 2017 McDonald criteria because, in this population, there is a higher probability that the first neurological event related to MS can have a picture of ADEM. Therefore, it is of utmost importance to emphasize that in the Franch cohort, the 2017 McDonald criteria were validated, and it was demonstrated that they could also be used in children below the age of 12 who had not had an ADEM presentation (4). Thus, for children below the age of 12, after excluding ADEM patients, the 2017 McDonald criteria have acceptable sensitivity and specificity. Conversely, it is important to recognize that including ADEM patients significantly reduces sensitivity and specificity.

The diagnostic algorithm involves the integration of all typical clinical and paraclinical characteristics that form the basis of a typical clinical picture (30). The main paraclinical indicators in POMS are brain and spinal cord MRI and, additionally, CSF examination (29). Clinical and paraclinical features should confirm dissemination in space and time to establish a diagnosis, and it is also necessary to exclude all other CNS diseases of differential diagnostic importance. MRI in the pediatric population enables quick and simple confirmation of dissemination in time and space and, thus, rapid diagnosis. Characteristic predilection localizations of changes on MRI are periventricular white matter, deep white matter,

Table 2. The 2017 McDonald criteria for dissemination in space (DIS) and time (DIT)

DIS demonstration requires one or more T2 lesions in at least 2 of 4 areas of the CNS: - Periventricular - Juxtacortical/cortical - Infratentorial - Spinal cord
DIT can be defined by one of the following: - A new T2 and/or gadolinium-enhancing lesion(s) on a follow-up MRI, compared to a baseline scan, irrespective of the timing of the baseline MRI - Simultaneous presence of gadolinium-enhancing and non-enhancing lesions at any time - CSF-specific oligoclonal bands

CNS central nervous system; MRI magnetic resonance imaging; CSF cerebrospinal fluid;
Adapted from Thompson et al. (29)

juxtacortical regions, corpus callosum, and infratentorial regions of the brain (brainstem and cerebellum), as well as changes in the spinal cord, optic nerve, and cortical lesions (31, 32). It must be emphasized that infratentorial and contrast-enhancing lesions at baseline MRI scans are more frequent in children than in adult-onset MS (4).

Numerous diagnostic criteria for POMS have been proposed throughout history. However, the first widely accepted official criteria emerged following the establishment of the International Multiple Sclerosis Study Group for children and adolescents (33). The majority of clinicians have relied on the criteria established by the International Study Group for Multiple Sclerosis in Children (28). It is necessary to monitor and follow the diagnostic protocol in order to make an accurate diagnosis of the disease.

Brain MRI lesions in POMS are typically small (less than 1 cm) ovoid-shaped areas with sharp borders and a homogeneous hyperintensity on T2-weighted sequences of at least 3mm in the long axis (34, 35). It is important to remember that these lesions usually appear larger because of the inflammatory edema, which is usually huge in children. Demyelinating lesions are present in both white and gray matter, especially in the cortical regions and deep gray matter (36).

Periventricular lesions are T2-hyperintense cerebral white matter lesions bordering the lateral ventricles without white matter in between, including the corpus callosum. The FLAIR sequences are the first choice for their detection because they show abnormalities even when standard T2-weighted images are normal (34, 37). It is important to emphasize that periventricular lesions are present in 86% of children with MS (38). Similarly, brainstem lesions are frequently present in 61% of POMS, significantly more often than in subjects with monophasic ADS (38). In total, infratentorial lesions are almost 25% more frequent in POMS compared to adult-onset MS (39).

Spinal cord lesions are hyperintense lesions, which show well-defined margins and are typically located in the cervical region, extending up to two vertebrae (40, 41). As already mentioned, POMS present a more elevated inflammatory component as adult-onset MS. Thus, contrast-enhancing lesions at baseline MRI scans are present at up to 70% of POMS (38). In contrast, enhancing lesions are present in only 10% of children with monophasic ADS (38).

Finally, very recently the group of experts recommended the following regarding MRI in POMS: a) The same standardized brain and spinal cord MRI protocols should be used for POMS as in adult-onset MS; b) In order to exclude non-MS diagnosis at onset, Gd-enhanced images are useful; c) For children with spinal cord manifestations or with inconclusive brain MRI, it is indicated to perform complete spinal cord MRI; d) Spinal cord MRI at baseline could be useful at baseline for all POMS; e) An innovative optic nerve MRI protocol is not recommended in POMS (42).

DIFFERENTIAL DIAGNOSIS

As already mentioned, ADS includes several CNS inflammatory conditions, such as ADEM, MS, MOGAD, and AQP4+ NMOSD. Although their clinical presentation and MRI findings may have certain similarities, it is important to mention that, for example, MOGAD is more common prepuberally than MS. Thus, presentation before the age of 11 speaks instead in favor of MOGAD, being a red flag of MS. Advancements in serological testing, recent development of cell-based assays for AQP-4 IgG and MOG-IgG have significantly improved differential diagnosis between MOGAD and AQP-4+NMOSD, and MS (43, 44).

A key consideration in the differential diagnosis of a potential POMS onset attack is acute ADEM, an inflammatory demyelinating disorder of the CNS that primarily affects children. It is characterized by polyfocal symptoms and encephalopathy, which are associated with typical MRI findings. ADEM is rare, but 0.07 to 0.9 per 100,000 children are affected by this disorder every year (19). Although it can affect people at any age, ADEM is more common in children, with a median age of onset at the age of 5 to 8. A male preponderance had been shown in most studies, with a male-to-female ratio ranging from 1:0.8 to 2.3:1 (19). In the last decade, to characterize the range of ADS, the International Pediatric Multiple Sclerosis Study Group (IPMSSG) produced consensus clinical and radiologic diagnostic criteria defining ADEM as an ADS (Table 3) (28). A key development in recent years is the recognition of the association of MOG-IgG with ADEM, as well as with multiphasic ADEM (MDEM) and ADEM followed by recurrent optic neuritis (ADEM-ON) (Table 3) (45, 46, 47). ADEM is very rarely the first manifestation of MS or NMOSD (less than 10%) (45).

Table 3. Criteria for acute disseminated encephalomyelitis (ADEM) and relapsing disorders following ADEM

ADEM	• Single polyfocal clinical CNS event with a presumed inflammatory cause • Encephalopathy that cannot be explained by fever, with MRI which typically presents with diffuse, poorly limited, large >1–2 cm lesions predominantly involving cerebral white matter; T1 hypointense white matter lesions are very rare; deep gray matter lesions (e.g., thalamus or basal ganglia) can be present • No new symptoms, signs, or MRI findings after three months of initial presentation of ADEM
Multiphasic ADEM (MDEM)	New event of ADEM three months or more after the initial event that can be associated with new or re-emergence of prior clinical and MRI findings
ADEM-ON	At least one subsequent attack of optic neuritis, without encephalopathy, with potential other neurological manifestations at least three months after initial ADEM
ADEM-MS	ADEM is followed three months later by a non-encephalopathic clinical event with new lesions on brain MRI consistent with MS; very rare
ADEM-NMOSD	ADEM is followed three months later by ON, myelitis, or area postrema syndrome, fulfilling NMOSD diagnostic criteria, commonly AQP4-IgG negative

ADEM, acute disseminated encephalomyelitis; CNS, central nervous system; ON, optic neuritis; MRI, magnetic resonance imaging; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; AQP4, aquaporin 4. Adapted from Pohl et al. (45).

CONCLUSION

MS is a chronic, neuroinflammatory, and neurodegenerative disease of CNS that rarely affects children in whom the onset can occur prior to the age of 18. The differential diagnosis of POMS can be broad. Thus, it may pose diagnostic dilemmas, especially at the initial presentation. Acquired demyelinating disorders, such as ADEM, NMOSD, and MOGAD, which are currently precisely defined unique disorders, continue to overlap with MS. This is due to certain similarities regarding clinical presentation and MRI. Early and accurate diagnosis is crucial, as it enables the prompt initiation of effective treatment.

Author Contributions

JD, JJ, BN, TP, and SM conceived and wrote the paper, revised it for important intellectual content, and approved the final submission.

References

1. Margoni M, Preziosa P, Rocca MA, Filippi M. Pediatric multiple sclerosis: developments in timely diagnosis and prognostication. Expert Rev Neurother 2022; 22(5): 393-403. doi: 10.1080/14737175.2022.2064743.
2. Hua LH, Solomon AJ, Tenembaum S, Scalfari A, Rovira À, Rostasy K, Newsome SD, Marrie RA, Magyari M, Kantarci O, Hemmer B, Hemingway C, Harnegie MP, Graves JS, Cohen JA, Bove R, Banwell B, Corboy JR, Waubant E. Differential Diagnosis of Suspected Multiple Sclerosis in Pediatric and Late-Onset Populations: A Review. JAMA Neurol 2024 Sep 16. doi: 10.1001/jamaneurol.2024.3062.
3. Yan K, Balijepalli C, Desai K, Gullapalli L, Druyts E. Epidemiology of pediatric multiple sclerosis: A systematic literature review and meta-analysis. Mult Scler Relat Disord 2020; 44: 102260. doi: 10.1016/j.msard.2020.102260.
4. Barraza G, Deiva K, Husson B, Adamsbaum C. Imaging in Pediatric Multiple Sclerosis: An Iconographic Review. Clin Neuroradiol 2021; 31(1): 61-71. doi: 10.1007/s00062-020-00929-8.
5. Brownlee WJ, Hardy TA, Fazekas F, Miller DH. Diagnosis of multiple sclerosis: progress and challenges. Lancet 2017; 389(10076): 1336-1346. doi: 10.1016/S0140-6736(16)30959-X.
6. Nikolić B, Ivančević N, Zaletel I, Rovčanin B, Samardžić J, Jančić J. Characteristics of pediatric multiple sclerosis: A tertiary referral center study. PLoS One 2020; 15(12): e0243031. doi: 10.1371/journal.pone.0243031.
7. Inaloo S, Haghbin S. Multiple sclerosis in children. Iran J Child Neurol 2013; 7(2): 1-10.
8. Langille MM, Islam T, Burnett M, Amezcua L. Clinical Characteristics of PediatricOnset and Adult-Onset Multiple Sclerosis in Hispanic Americans. J Child Neurol 2016; 31(8): 1068-73. doi: 10.1177/0883073816638754.
9. Renoux C, Vukusic S, Mikaeloff Y, Edan G, Clanet M, Dubois B, Debouverie M, Brochet B, Lebrun-Frenay C, Pelletier J, Moreau T, Lubetzki C, Vermersch P, Roullet E, Magy L, Tardieu M, Suissa S, Confavreux C; Adult Neurology Departments KIDMUS Study Group. Natural history of multiple sclerosis with childhood onset. N Engl J Med 2007; 356(25): 2603-13. doi: 10.1056/NEJMoa067597.
10. Chitnis T, Aaen G, Belman A, Benson L, Gorman M, Goyal MS, Graves JS, Harris Y, Krupp L, Lotze T, Mar S, Ness J, Rensel M, Schreiner T, Tillema JM, Waubant E, Weinstock-Guttman B, Roalstad S, Rose J, Weiner HL, Casper TC, Rodriguez M; US Network of Paediatric Multiple Sclerosis Centers. Improved relapse recovery in paediatric compared to adult multiple sclerosis. Brain 2020; 143(9): 2733-2741. doi: 10.1093/brain/awaa199.
11. Waldman A, Ness J, Pohl D, Simone IL, Anlar B, Amato MP, Ghezzi A. Pediatric multiple sclerosis: Clinical features and outcome. Neurology 2016; 87(9 Suppl 2): S74-81. doi: 10.1212/WNL.0000000000003028.
12. Jeong A, Oleske DM, Holman J. Epidemiology of Pediatric-Onset Multiple Sclerosis: A Systematic Review of the Literature. J Child Neurol 2019; 34(12): 705-712. doi: 10.1177/0883073819845827.
13. Harding KE, Liang K, Cossburn MD, Ingram G, Hirst CL, Pickersgill TP, Te Water Naude J, Wardle M, Ben-Shlomo Y, Robertson NP. Long-term outcome of paediatric-onset multiple sclerosis: a population-based study. J Neurol Neurosurg Psychiatry 2013; 84(2): 141-7. doi: 10.1136/jnnp-2012-303996.
14. Bartels F, Nobis K, Cooper G, Wendel E, Cleaveland R, Bajer-Kornek B, Blaschek A, Schimmel M, Blankenburg M, Baumann M, Karenfort M, Finke C, Rostásy K. Childhood multiple sclerosis is associated with reduced brain volumes at first clinical presentation and brain growth failure. Mult Scler 2019; 25(7): 927-936. doi: 10.1177/1352458519829698.
15. Wallach AI, Waltz M, Casper TC, Aaen G, Belman A, Benson L, Chitnis T, Gorman M, Graves J, Harris Y, Lotze TE, Mar S, Moodley M, Ness JM, Rensel M, Rodriguez M, Rose JW, Schreiner T, Tillema JM, Waubant E, Weinstock-Guttman B, Charvet LE, Krupp LB. Cognitive processing speed in pediatric-onset multiple sclerosis: Base-

line characteristics of impairment and prediction of decline. Mult Scler 2020; 26(14): 1938-1947. doi: 10.1177/1352458519891984.

16. McKay KA, Friberg E, Razaz N, Alexanderson K, Hillert J. Long-term Socioeconomic Outcomes Associated With Pediatric-Onset Multiple Sclerosis. JAMA Neurol 2021; 78(4): 478-482. doi: 10.1001/jamaneurol.2020.5520.
17. Eyre M, Absoud M, Abdel-Mannan O, Crichton S, Hacohen Y, Rossor T, Rudebeck S, Giovannoni G, Lim M, Hemingway C. Academic outcomes before and after clinical onset of acquired demyelinating syndromes in children: a matched cohort data linkage study. Ann Clin Transl Neurol 2024; 11(11): 3025-3030. doi: 10.1002/acn3.52198.
18. Baroncini D, Simone M, Iaffaldano P, Brescia Morra V, Lanzillo R, Filippi M, Romeo M, Patti F, Chisari CG, Cocco E, Fenu G, Salemi G, Ragonese P, Inglese M, Cellerino M, Margari L, Comi G, Zaffaroni M, Ghezzi A; Italian MS registry. Risk of Persistent Disability in Patients With Pediatric-Onset Multiple Sclerosis. JAMA Neurol 2021; 78(6): 726-735. doi: 10.1001/jamaneurol.2021.1008.
19. Paolilo RB, Deiva K, Neuteboom R, Rostásy K, Lim M. Acute Disseminated Encephalomyelitis: Current Perspectives. Children (Basel) 2020; 7(11): 210. doi: 10.3390/children7110210.
20. Fadda G, Armangue T, Hacohen Y, Chitnis T, Banwell B. Paediatric multiple sclerosis and antibody-associated demyelination: clinical, imaging, and biological considerations for diagnosis and care. Lancet Neurol 2021; 20(2): 136-149. doi: 10.1016/S1474-4422(20)30432-4.
21. Fadda G, Brown RA, Longoni G, Castro DA, O'Mahony J, Verhey LH, Branson HM, Waters P, Bar-Or A, Marrie RA, Yeh EA, Narayanan S, Arnold DL, Banwell B; Canadian Pediatric Demyelinating Disease Network. MRI and laboratory features and the performance of international criteria in the diagnosis of multiple sclerosis in children and adolescents: a prospective cohort study. Lancet Child Adolesc Health 2018; 2(3): 191-204. doi: 10.1016/S2352-4642(18)30026-9.
22. Pereira FV, Jarry VM, Castro JTS, Appenzeller S, Reis F. Pediatric inflammatory demyelinating disorders and mimickers: How to differentiate with MRI? Autoimmun Rev 2021; 20(5): 102801. doi: 10.1016/j.autrev.2021.102801.
23. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol 2015; 15(9): 545-58. doi: 10.1038/nri3871.
24. Schmierer K, McDowell A, Petrova N, Carassiti D, Thomas D.L, Miquel M.E, Quantifying multiple sclerosis pathology in post mortem spinal cord using MRI. NeuroImage 2018; 182: 251-258. doi. org/10.1016/j.neuroimage.2018.01.052.
25. Aliyu M, Zohora FT, Ceylan A, Hossain F, Yazdani R, Azizi G. Immunopathogenesis of multiple sclerosis: molecular and cellular mechanisms and new immunotherapeutic approaches. Immunopharmacol Immunotoxicol 2024; 46(3): 355-377. doi: 10.1080/08923973.2024.2330642.
26. Alroughani R, Boyko A. Pediatric multiple sclerosis: a review. BMC Neurol 2018; 18(1): 27. doi:10.1186/s12883-018-1026-3.
27. Pfeifenbring S, Bunyan RF, Metz I, Röver C, Huppke P, Gärtner J, Lucchinetti CF, Brück W. Extensive acute axonal damage in pediatric multiple sclerosis lesions. Ann Neurol 2015; 77(4): 655-67. doi: 10.1002/ana.24364.
28. Krupp LB, Tardieu M, Amato MP, Banwell B, Chitnis T, Dale RC, Ghezzi A, Hintzen R, Kornberg A, Pohl D, Rostasy K, Tenembaum S, Wassmer E; International Pediatric Multiple Sclerosis Study Group. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. Mult Scler 2013; 19(10): 1261-7. doi: 10.1177/1352458513484547.
29. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS, Fujihara K, Galetta SL, Hartung HP, Kappos L, Lublin FD, Marrie RA, Miller AE, Miller DH, Montalban X, Mowry EM, Sorensen PS, Tintoré M, Traboulsee AL, Trojano M, Uitdehaag BMJ, Vukusic S, Waubant E, Weinshenker BG, Reingold SC, Cohen JA. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018; 17(2): 162-173. doi: 10.1016/S1474-4422(17)30470-2.
30. Nikolic B, Zaletel I, Ivancevic N, Rovcanin B, Pepic A, Samardzic J, Jancic J. The usefulness of visual evoked potentials in the assessment of the pediatric multiple sclerosis. Eur J Paediatr Neurol 2022; 36: 130-136. doi: 10.1016/j.ejpn.2021.12.005.
31. Filippi M, Rocca MA, Arnold DL, Bakshi R, Barkhof F, De Stefano N, Fazekas F, Frohman E, Wolinsky JS. EFNS guidelines on the use of neuroimaging in the management of multiple sclerosis. Eur J Neurol 2006; 13(4): 313-25. doi: 10.1111/j.1468-1331.2006.01543.x.
32. Mesaros S, Rocca MA, Absinta M, Ghezzi A, Milani N, Moiola L, Veggiotti P, Comi G, Filippi M. Evidence of thalamic gray matter loss in pediatric multiple sclerosis. Neurology 2008; 70(13 Pt 2): 1107-12. doi: 10.1212/01.wnl.0000291010.54692.85.
33. Krupp LB, Banwell B, Tenembaum S; International Pediatric MS Study Group. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. Neurology 2007; 68(16 Suppl 2): S7-12. doi: 10.1212/01.wnl.0000259422.44235.a8.
34. Rovira À, Wattjes MP, Tintoré M, Tur C, Yousry TA, Sormani MP, De Stefano N, Filippi M, Auger C, Rocca MA, Barkhof F, Fazekas F, Kappos L, Polman C, Miller D, Montalban X; MAGNIMS study group. Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis-clinical implementation in the diagnostic process. Nat Rev Neurol 2015; 11(8): 471-82. doi: 10.1038/nrneurol.2015.106.
35. Fazekas F, Barkhof F, Filippi M, Grossman RI, Li DK, McDonald WI, McFarland HF, Paty DW, Simon JH, Wolinsky JS, Miller DH. The contribution of magnetic resonance imaging to the diagnosis of multiple sclerosis. Neurology 1999; 53(3): 448-56. doi: 10.1212/wnl.53.3.448.
36. Bar-Or A, Hintzen RQ, Dale RC, Rostasy K, Brück W, Chitnis T. Immunopathophysiology of pediatric CNS inflammatory demyelinating diseases. Neurology 2016; 87(9 Suppl 2): S12-9. doi: 10.1212/WNL.0000000000002821.
37. Filippi M, Rocca MA, Ciccarelli O, De Stefano N, Evangelou N, Kappos L, Rovira A, Sastre-Garriga J, Tintorè M, Frederiksen JL, Gasperini C, Palace J, Reich DS, Banwell B, Montalban X, Barkhof F; MAGNIMS Study Group. MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. Lancet Neurol 2016; 15(3): 292-303. doi: 10.1016/S1474-4422(15)00393-2.
38. Verhey LH, Branson HM, Shroff MM, Callen DJ, Sled JG, Narayanan S, Sadovnick AD, Bar-Or A, Arnold DL, Marrie RA, Banwell B; Canadian Pediatric Demyelinating Disease Network. MRI parameters for prediction of multiple sclerosis diagnosis in children with acute CNS demyelination: a prospective national cohort study. Lancet Neurol 2011; 10(12): 1065-73. doi: 10.1016/S1474-4422(11)70250-2.
39. Gorman MP, Healy BC, Polgar-Turcsanyi M, Chitnis T. Increased relapse rate in pediatric-onset compared with adult-onset multiple sclerosis. Arch Neurol 2009; 66(1): 54-9. doi: 10.1001/archneurol.2008.505.
40. Simon JH, Li D, Traboulsee A, Coyle PK, Arnold DL, Barkhof F, Frank JA, Grossman R, Paty DW, Radue EW, Wolinsky JS. Standardized MR imaging protocol for multiple sclerosis: Consortium of MS Centers consensus guidelines. AJNR Am J Neuroradiol 2006; 27(2): 455-61.
41. Hua LH, Donlon SL, Sobhanian MJ, Portner SM, Okuda DT. Thoracic spinal cord lesions are influenced by the degree of cervical spine involvement in multiple sclerosis. Spinal Cord 2015; 53(7): 520-5. doi: 10.1038/sc.2014.238.
42. Rocca MA, Preziosa P, Barkhof F, Brownlee W, Calabrese M, De Stefano N, Granziera C, Ropele S, Toosy AT, Vidal-Jordana À, Di Filippo M, Filippi M. Current and future role of MRI in the diagnosis and prognosis of multiple sclerosis. Lancet Reg Health Eur 2024; 44: 100978. doi: 10.1016/j.lanepe.2024.100978.
43. Mader S, Gredler V, Schanda K, Rostasy K, Dujmovic I, Pfaller K, Lutterotti A, Jarius S, Di Pauli F, Kuenz B, Ehling R, Hegen H, Deisenhammer F, Aboul-Enein F, Storch MK, Koson P, Drulovic J, Kristoferitsch W, Berger T, Reindl M. Complement activating antibodies to myelin oligodendrocyte glycoprotein in neuromyelitis optica and related disorders. J Neuroinflammation 2011; 8: 184. doi: 10.1186/1742-2094-8-184.
44. Pekmezovic T, Jovicevic V, Andabaka M, Momcilovic N, Veselinovic N, Tamas O, Budmkic M, Todorovic S, Jeremic M, Dincic E, Vojinovic S, Andrejevic S, Mesaros S, Drulovic J. Aquaporin4-IgG seropositivity significantly increases the risk of comorbid autoimmune

diseases in NMOSD patients: population-based registry data. J Neurol 2024; 271(12): 7525-7536. doi: 10.1007/s00415-024-12698-2.

45. Pohl D, Alper G, Van Haren K, Kornberg AJ, Lucchinetti CF, Tenembaum S, Belman AL. Acute disseminated encephalomyelitis: Updates on an inflammatory CNS syndrome. Neurology 2016; 87(9 Suppl 2): S38-45. doi: 10.1212/WNL.0000000000002825.
46. Wong YYM, Hacohen Y, Armangue T, Wassmer E, Verhelst H, Hemingway C, van Pelt ED, Catsman-Berrevoets CE, Hintzen RQ, Deiva K, Lim MJ, Rostásy K, Neuteboom RF. Paediatric acute disseminated encephalomyelitis followed by optic neuritis: disease course, treatment response and outcome. Eur J Neurol 2018; 25(5): 782-786. doi: 10.1111/ene.13602.
47. Hacohen Y, Mankad K, Chong WK, Barkhof F, Vincent A, Lim M, Wassmer E, Ciccarelli O, Hemingway C. Diagnostic algorithm for relapsing acquired demyelinating syndromes in children. Neurology 2017; 89(3): 269-278. doi: 10.1212/WNL.0000000000004117.

DILEME U DIFERENCIJALNOJ DIJAGNOZI PEDIJATRIJSKE MULTIPLE SKLEROZE

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Sažetak

Multipla skleroza (MS) je hronična, inflamatorna i neurodegenerativna bolest centralnog nervnog sistema (CNS). Obično se javlja u ranom ili srednjem odraslom dobu, a MS sa pedijatrijskim početkom (POMS), definisan prvim napadom MS koji se javlja pre 18. godine, je manje čest. Aktuelni podaci iz Danskog registra multiple skleroze pokazuju da je skoro 3% pacijenata imalo početak pre 18. godine. U poređenju sa MS kod odraslih, pacijenti sa POMS-om obično imaju aktivniji tok bolesti, što dovodi do češćih relapsa, ali sporijeg dugoročnog pogoršanja onesposobljenosti. Kod POMS-a, dijagnostičke dileme mogu uključivati razlikovanje MS od akutnog diseminovanog encefalomijelitisa (ADEM) i bolesti povezane sa antitelom na mijelina oligodendrocitni glikoprotein (MOGAD), od kojih se oba najčešće javljaju kod dece i imaju slične pojedine kliničke i radiološke karakteristike u poređenju sa MS. Nasledne leukodistrofije se takođe mogu uzeti u obzir u diferencijalnoj dijagnozi, ali samo u određenim slučajevima, jer je njihova klinička karakteristika progresivan tok bolesti, neuobičajen za druge gore navedene stečene demijelinizacione sindrome, uključujući bolesti iz spektra neuromijelitisa optika. Treba imati na umu da je magnetna rezonanca mozga i kičmene moždine ključna za postavljanje pravovremene i tačne dijagnoze POMS, nakon isključivanja alternativnih dijagnoza, što omogućava hitan početak efektivnog lečenja.

Ključne reči: multipla skleroza, dijagnoza, početak bolesti u detinjstvu

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REVIEW ARTICLE

Techniques for sentinel lymph node biopsy in breast cancer patients

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Summary

Breast cancer is the most frequent cancer among women worldwide. Cancer cells primarily spread through the lymphatic vessels, with axillary lymph node status being one of the most reliable prognostic factors in breast cancer patients. Axillary lymph node staging in breast cancer patients was initially performed by axillary lymph node dissection. Sentinel lymph node biopsy is the standard of care in clinically and radiologically negative axilla.

According to the current guidelines, double contrast is a recommended procedure for identifying the sentinel lymph node. Nowadays, several new tracers are in clinical practice with a high identification rate and low false negative results. Due to technological and resource limitations, many medical centers worldwide lack the facilities to apply new radioactive tracers. The use of blue dye alone is a reliable and effective diagnostic and surgical technique, offering a high identification rate and low false-negative results.

Key words: breast cancer, biopsy, sentinel lymph node

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INTRODUCTION

According to The Cancer Registry of the Republic of Serbia, breast cancer (BC) is the most common cancer affecting women in Serbia. Every year, 4,600 women get diagnosed with BC, and 1,600 women die from this disease. Every eighth woman in Serbia is diagnosed with BC (1).

Breast cancer is a heterogeneous disease with multiple subtypes. A fundamental step in breast cancer progression and metastasis is the invasion of the basal layer (2). There are several classifications of BC. The fundamental classification involves the histological categorization of BC. Invasive ductal carcinoma is the most common histological subtype, accounting for 70%–80% of all invasive breast carcinomas, followed by invasive lobular carcinoma at approximately 10%. The remaining cases are rarer subtypes, including mucinous, cribriform, papillary, tubular, medullary, metaplastic, and apocrine carcinomas (3). The immunohistochemical classification is the most widely used method for defining the molecular subtypes of breast cancer. Immunohistochemistry assesses the presence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor 2 (Her2). Based on the expression of these receptors, breast cancer is classified into the following subtypes: Luminal A, Luminal B, Her2 positive, and triple negative. The molecular classification of BC holds significant clinical importance as different subtypes exhibit distinct characteristics and prognoses, thereby guiding oncological treatment based on molecular subtypes (4,5).

Diagnostic and staging process of breast cancer is based on physical examination, ultrasound, mammography, and magnetic resonance imaging of the breast (6,7). It is important in terms of diagnosis of the primary tumor as well as the evaluation of the stage of axillary disease.

One of the most accurate prognostic factors in breast cancer is the presence of axillary lymph node metastasis (8). Detailed knowledge of the anatomy of the lymphatic system of upper extremities and the breast is fundamental in the adequate assessment of the axillary lymphatic status (9).

Axillary lymph node staging in breast cancer patients was initially performed by axillary lymph node dissection. This technique was accurate, but it had significant morbidities such as upper extremity numbness, surgical site and surrounding soft tissue infection, and lymphedema of the unilateral upper extremity. (10) Sentinel node biopsy for breast cancer was introduced by Krag in 1993 (11). The sentinel lymph node (SLN) is the first to receive drainage directly from the tumor. Sentinel lymph node biopsy (SLNB) is a minimally invasive technique to confirm regional lymph node metastasis in cancer patients. SLN biopsy is the gold standard for axillary lymph node metastasis assessment in patients with clinically and radiologically negative axilla (N0 status) (12).

Among the biological subtypes of breast cancer, positive lymph nodes were most frequently observed in luminal cancer, while the lowest frequency was found in triple-negative breast cancer (13)

The most frequent localization of the sentinel node is the lower medial part of the axilla alongside the lateral thoracic vein, below the second intercostobrachial nerve (87%) or above the nerve (11.5%) (14).

The most commonly used SLNB techniques are dual-the modality method with radioactive isotope technetium-99m (Tc)- labeled nano colloid and blue dye, blue dye alone, radioactive isotope technetium-99m (Tc)- labeled nano colloid alone, indocyanine green fluorescence, super-paramagnetic iron oxide (SPIO) nanoparticles, contrast-enhanced ultrasound imaging using microbubble (CEUS) (15).

Dual-modality method with radioactive isotope technetium-99m (Tc)- labeled nano colloid and blue dye

The dual-modality method with radioactive isotope and blue-dye method are considered the gold standard for SLNB. This is an expensive method that requires a nuclear medicine facility; thus, it is not available in every hospital. (16)

Sentinel lymph node biopsy technique using radioactive isotope is conducted through the preoperative administration of a radioisotope solution, specifically 99mTc colloidal, injected into the subareolar region of the breast or the peritumoral area. Following this injection, lymphoscintigraphy is performed a few hours later using a gamma camera to visualize the lymphatic drainage. Surgical intervention is generally scheduled for the day after lymphoscintigraphy. The procedure begins with injecting the blue dye into the same anatomical location as the radioactive isotope. During this procedure, a handheld intraoperative probe is used to identify radioactive sentinel lymph nodes. Subsequently, all labeled or stained nodes are excised individually and subjected to pathological examination for further analysis (10,12). Disadvantages of using radioactive isotopes for SLN marking include the need for preoperative injection hours before surgery, reliance on nuclear medicine personnel, and exposure to radiation for both patients and healthcare staff. Lymphoscintigraphy does not provide real-time visualization of lymph nodes; once the gamma camera is removed, surgeons dissect areas previously identified as radioactively hot (17).

The identification of the dual-modality method with a radioactive isotope and blue dye is up to 99.6% (18).

Blue dye alone

In many countries, the dual modality approach for sentinel lymph node biopsy (SLNB) is not feasible; therefore,

the use of blue dye alone is a prevalent alternative for performing SLNB (5 Zhou). The application of blue dye is considered acceptable and can be effectively utilized in institutions with limited access to nuclear tracers (9, 19).

The procedure is performed in an operating room setting. Following the administration of general endotracheal anesthesia, 2 mL of methylene blue is injected subdermally around the areola and peritumorally, accompanied by a brief massage to facilitate lymphatic drainage from the breast. Access to the axilla is achieved through a transverse incision, and upon the identification of the sentinel lymph node (SLN), it is excised for ex tempore histopathological evaluation.

The SLNB procedure is recognized as safe and accurate and recommended when nuclear tracers or alternative modalities are unavailable. This method demonstrated an identification rate of 94.74% (8, 20).

Indocyanine green fluorescence

Indocyanine green (ICG) is used to assess liver function and cardiac output and to monitor free flap perfusion. Unlike fluorescein, ICG is entirely bound to plasma proteins and fluoresces in the near-infrared spectrum. This fluorochrome absorbs light at approximately 800 nm, emitting a fluorescent signal when subatomic particles transition from an excited state to a ground state.

Indocyanine green fluorescence is one of the most well-known alternative methods for SLN localization. It offers a comparable SLN detection rate to radioisotope or blue dye. The procedure is performed with a 1-5 mL volume of ICG, administered subdermally or intradermally into the retroareolar or periareolar breast tissue. The breast tissue is then massaged for 2 minutes to facilitate distribution (21, 22).

The fluorescence is not visually detectable; therefore, the operating room lights are typically dimmed, and the Photodynamic Eye (PDE: Hamamatsu Photonics, Hamamatsu, Japan) system is used to capture the black-and-white images of fluorescent lymphatics and sentinel nodes on a monitor. ICG administration is contraindicated in patients with iodine allergies due to the presence of sodium iodide. Transcutaneous fluorescence of lymphatic vessels aids in identifying the location of axillary incision. After the incision, sentinel nodes are localized using an infrared torch PDE and excised. This technique may be used independently or in combination with blue dye or radioisotope techniques (22).

Superparamagnetic iron oxide (SPIO) nanoparticles

Supermagnetic iron oxide (SPIO) nanoparticles are used as contrast agents in magnetic resonance imaging (MRI). Sienna+® (Endomagnetics Ltd, Cambridge, UK) is a brown liquid SPIO tracer with an average particle size of 60 nm and has been used for sentinel node mapping. To prepare the solution, 2 mL of Sienna is diluted with 5 mL of saline and injected subcutaneously in the breast. Following injection, the site is massaged for approximately five minutes to facilitate the movement of SPIO nanoparticles through the lymphatic system, where they accumulate in the sentinel lymph node. The sentinel node/s may stain brown and the tracer can be detected using the hand-held magnetometer- Sentimag® (Endomagnetics Ltd) magnetometer probe, which functions similarly to a gamma probe for radioisotope detection after a migration period of 20 minutes. It is important to note that metal instruments can disrupt the ferromagnetic signal, and these need to be removed or replaced with plastic alternatives during localization. This technique is contraindicated in individuals with allergies to iron or dextran compounds, iron overload diseases, pacemakers, or ferrous metal-containing devices in the chest wall (22,23).

Detection rates of sentinel nodes using this method range from 94.4 to 98%.

The SPIO tracer is not radioactive, which simplifies implementation by avoiding the regulatory challenges associated with radioisotopes. It is available in approximately 30 countries, and reassuringly, data from multiple studies indicate that its detection rates are non-inferior to those of standard trace techniques (22).

Contrast-enhanced ultrasound imaging using microbubbles (CEUS)

Contrast-enhanced ultrasound (CEUS) utilizing microbubbles dispersed in sulfur hexafluoride gas, injected intradermally around the areola, was first documented in 2013 for sentinel lymph node biopsy. This technique employs an ultrasound contrast agent in conjunction with a contrast-specific mode of the ultrasound machine, allowing dynamic contrast-enhanced ultrasound imaging to facilitate non-operative identification and biopsy of sentinel nodes.

Second-generation ultrasound contrast agents comprise microbubbles filled with various gases encased in a lipid shell. While no adverse effects have been reported in breast applications, these agents may be perceived as foreign by the immune system, which raises the possibility of hypersensitivity reactions. The contrast agent is administered intradermally or subdermally at the upper outer periareolar region or directly under the areola. Following injection, the breast is gently massaged for several seconds. Visualization of the lymphatic channels is achieved through contrast pulse sequencing, enabling tracking into the axilla to locate the sentinel node that accumulates the contrast agent.

Repeated injections may be performed in cases where localization fails. This raises safety concerns regarding potential damage to the microvasculature. After successful localization, fine needle aspiration or core needle biopsy of the draining node is conducted using conventional grayscale ultrasound to assess axillary staging (24).

The CEUS procedure offers several benefits, including the elimination of radioisotope use, avoiding potential irradiation, eliminating the necessity for a nuclear medicine facility, providing real-time visualization of sentinel lymph nodes (SLN), and utilizing a low-cost contrast agent. However, drawbacks of this technique include extended procedure duration relative to alternative methods, the necessity for proficiency in axillary ultrasound examination, a prolonged learning curve, and dependence on operator skill. CEUS has a lower detection rate and sensitivity compared to the blue dye method (24). The Ceus detection rate is 96.3% (25).

CT lymphography (CTLG)

Computed tomography lymphography offers a novel way of localizing SLN, with possible combinations with other techniques (ICG fluorescence) allowing for a non-invasive highly accurate localization process compared to the radioisotope/ blue-dye method (21).

CTLG is a method in which computed tomography (CT) scans are obtained after local injection of a mixture of iodine contrast and local anesthetic into the areola to identify contrast-enhanced lymphatic vessels and sentinel lymph nodes. Contiguous 2-mm-thick CT images are obtained 3 minutes after the massage from the upper thorax to the axilla. After detection of the SLN –which is the LN enhanced with the dye-, its location can be mapped on the skin by marking the point of crossing of the vertical & horizontal lines of the red laser light beam of the CT machine. That laser beam is moved according to the SLN site on the CT. CTLG was performed 1–2 days before surgery (26, 27).

During surgery, 1.5–3 ml of dye (blue dye, Indigo carmine, etc.) was injected into the same site. Sentinel lymph node biopsy was then performed, relying on the images and markings obtained on CTLG. The identification rate of sentinel lymph nodes with CTLG is 92.9% (26, 27).

CONCLUSION

SLN biopsy is the gold standard for axillary lymph node metastasis assessment in patients with clinically and radiologically negative axilla. The gold standard for SLNB is the Dual-modality method with radioactive isotope and blue-dye method. Applying blue dye alone is a reliable and effective procedure in institutions with limited access to nuclear tracers. There are some new tracers with high identification rate and low false negative results.

References

1. Institute of Public Health of Serbia „Dr Milan Jovanovic Batut“ Health statistical yearbook of Republic of Serbia. 2022, URL: https://www.batut.org.rs/download/publikacije/pub2022v1.pdf
2. Zdravkovic D, Ivanovic N, Crnokrak B. Microinvasion: could it be sufficient diagnostic criteria for the optimal treatment decision? Breast Cancer Res Treat. 2019 Aug;176(3):717. doi: 10.1007/s10549-019-05269-x.
3. Tsang, J. Y. S.; Tse, G. M. Molecular classification of breast cancer. Adv Anat Pathol. 2019; 27: 27–35. doi: 10.1097/PAP.0000000000000232.
4. Sørlie, T, Perou, C. M, Tibshirani, R, Aas, T, Geisler, S, Johnsen, H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001; 98: 10869–10874. doi: 10.1073/pnas.191367098.
5. Zdravkovic D, Nikolic D, Zdravkovic M. Heterogeneity of tumor cells and metastases in breast cancer patients: cause or consequence? Breast Cancer Res Treat. 2018;172(1):241. doi: 10.1007/s10549-018-4913-9.
6. Zdravkovic D, Granic M, Zdravkovic M, Randjelovic T, Nikolic D, Ivanovic N. Physical examination: the first step in sophisticated diagnostic path of breast cancer. World J Surg. 2013 Sep;37(9):2252-3. doi: 10.1007/s00268-013-2057-y.
7. Bjelica D, Colakovic N, Opric S, Zdravkovic D, Loboda B, Petricevic S, Gojgic M, Zecic O, Skuric Z, Zecic K, Ivanovic N. Non-Invasive 3D Breast Tumor Localization: A Viable Alternative to Invasive Tumor Marking. Cancers (Basel). 2024 Jul 17;16(14):2564. doi: 10.3390/cancers16142564.
8. Hermansyah D, Rahayu Y, Azrah A, Pricilia G, Paramita D, Siregar ES, et al. Accuracy of Methylene Blue Test as Single Technique for Sentinel Lymph Node Biopsy in Early Stages Breast Cancer. Asian Pac J Cancer Prev. 2021;22(9):2765-9. doi: 10.31557/apjcp.2021.22.9.2765.
9. Ivanovic N, Zdravkovic D, Granic M, Sredic B, Colakovic N, Stojiljkovic M. Lymph nodes behind the axillary neurovascular bundle: case report and cadaveric study of frequency and distribution. Surg Radiol Anat. 2015 Dec;37(10):1283-6. doi: 10.1007/s00276-015-1506-8.
10. Zhou Y, Li Y, Mao F, Zhang J, Zhu Q, Shen S, et al. Preliminary study of contrast-enhanced ultrasound in combination with blue dye vs. indocyanine green fluorescence, in combination with blue dye for sentinel lymph node biopsy in breast cancer. BMC Cancer. 2019;19(1). doi:10.1186/s12885-019-6165-4.
11. Krag DN, Weaver DL, Alex JC, Fairbank JT. Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surg Oncol. 1993;2(6):335-40. doi: 10.1016/0960-7404(93)90064-6.
12. Kedrzycki MS, Leiloglou M, Ashrafian H, Jiwa N, Thiruchelvam PT, Elson DS, Leff DR. Meta-analysis Comparing Fluorescence Imaging with Radioisotope and Blue Dye-Guided Sentinel Node Identification for Breast Cancer Surgery. Ann Surg Oncol. 2020. 28(7):3738-48. doi: 10.1245/s10434-020-09288-7.
13. Si C, Jin Y, Wang H, et al. Association between molecular subtypes and lymph node status in invasive breast cancer. Int J Clin Exp Pathol 2014;7:6800-6.
14. Clough KB, Nasr R, Nos C, Vieira M, Inguenault C, Poulet B. New ana tomical classification of the axilla with implications for sentinel node biopsy. Br J Surg. 2010;97:1659–65.
15. Mok CW, Tan S-, Zheng Q, Shi L. Network meta-analysis of novel and conventional sentinel lymph node biopsy techniques in breast cancer. BJS Open. 2019;3(4):445-52. doi: 10.1002/bjs5.50157.
16. Chavda J, Mishra A, Ashutosh Silodia, Sanjay Kumar Yadav, Deepti Bala Sharma, Sharma D, et al. Validation sentinel lymph node biopsy study in cN0 axilla using low-cost dual dye technique: potential solution for resource poor settings. Breast Cancer Res Treat. 2022;193(1):105–10. doi: 10.1007/s10549-022-06556-w.
17. White KP, Sinagra D, Dip F, Rosenthal RJ, Mueller EA, Lo Menzo E, et al. Indocyanine green fluorescence versus blue dye, technetium-99M, and the dual-marker combination of technetium-99M + blue dye for sentinel lymph node detection in early breast cancer-meta-analysis including consistency analysis. Surgery. 2024;175(4):963–73. doi: 10.1016/j.surg.2023.10.021.

18. Long Y, Qi Xiaowei, Yi Z, Xinhua Y, Fan Z, Fan Linjun, et al. Comparison of sentinel lymph node detection performances using blue dye in conjunction with indocyanine green or radioisotope in breast cancer patients: a prospective single-center randomized study. Cancer Biol Med. 2018;15(4):452–2. doi: 10.20892/j.issn.2095-3941.2018.0270.
19. Bongkodmas P, Vacharathit V, Lerttiendamrong B, Manasnayakorn S, Tantiphlachiva K, Vongwattanakit P, et al. New concept in selecting blue dye injection site effect on clinical outcome of early-stage breast cancer patients: a retrospective cohort. World J Surg Oncol. 2024;22(1). doi: 10.1186/s12957-024-03493-4.
20. Loboda B, Ivanović N, Granić M, Zdravković D, Čolaković N, Sredić B, et al. Sentinel lymph node identification with dye and intraoperative axillary palpation in breast cancer: Single institution experience. Eur J Surg Oncol. 2023;49(2):e88–9. doi: 10.1016/j.ejso.2022.11.272.
21. Subramonian S, Chopra S, Vidya R. New Alternative Techniques for Sentinel Lymph Node Biopsy. Medicina (Kaunas). 2023;59(12):2077–7. doi: 10.3390/medicina59122077.
22. Goyal A. New Technologies for Sentinel Lymph Node Detection. Breast Care (Basel). 2018;13(5):349-53. doi: 10.1159/000492436.
23. Rocco N, Nunzio Velotti, Pontillo M, Vitiello A, Berardi G, Accurso A, et al. New techniques versus standard mapping for sentinel lymph node biopsy in breast cancer: a systematic review and meta-analysis. Updates Surg. 2023;75(6):1699–710. doi: 10.1007/s13304-023-01560-1.
24. Mathelin C, Lodi M. Narrative review of sentinel lymph node biopsy in breast cancer: a technique in constant evolution with still numerous unresolved questions. Chin Clin Oncol. 2021;10(2):20–0. doi: 10.21037/cco-20-207.
25. Cui Q, Dai L, Li J, Shen Y, Tao H, Zhou X, et al. Contrast-enhanced ultrasound-guided sentinel lymph node biopsy in early-stage breast cancer: a prospective cohort study. World J Surg Oncol. 2023;21(1). doi: 10.1186/s12957-023-03024-7.
26. Hamdy O, Farouk O, El-Badrawy A, Denewer A, Setit A. Sentinel lymph node biopsy in breast cancer guided by CT lymphography; History, evolution and current applications. Breast Dis. 2021;40(4):219–25. doi: 10.3233/BD-201046.
27. Kamata A, Miyamae T, Koizumi M, Kohei H, Sarukawa H, Nemoto H, et al. Using Computed Tomography Lymphography for Mapping of Sentinel Lymph Nodes in Patients with Breast Cancer. J Clin Imaging Sci. 2021;11:43. doi: 10.25259/JCIS_33_2021.

TEHNIKE BIOPSIJE "STRAŽARSKOG" LIMFNOG NODUSA KOD PACIJENTKINJA SA KARCINOMOM DOJKE

Darko[1] Zdravković[,2], Barbara[2] Loboda, Milan[2] Gojgić, Borislav[1] Toškovic[,3]

Sažetak

Karcinom dojke je najčešća maligna bolest kod žena. Limfogeni put je primarni način širenja karcinoma dojke, što je slučaj i kod drugih karcinoma. Status regionalnih limfnih čvorova je jedan od najvažnijih prognostičkih parametara obolelih od karcinoma dojke. Aksilarna disekcija je bila primarni način procene aksilarnog statusa kod klinički i radiološki negativnih limfnih čvorova. Danas, biopsija stražarskih limfnih čvorova predstavlja proceduru izbora kod ovih pacijenata.

Prema važećim preporukama, metoda dvostrukog kontrasta (kombinacija radioaktivnog koloida i metilenskog plavog) predstavlja proceduru izbora. U novije vreme, u svakodnevnu praksu se uvode i drugi obeleživači. U velikom broju ustanova u svetu nema mogućnosti za korišćenje radioaktivnog koloida kao i novih obeleživača čija upotreba je povezana sa tehnološkim poteškoćama i materijalnim izdacima. Korišćenje metilenskog plavog kao jedinog kontrasta od strane obučenog hirurga predstavlja pouzdanu metodu sa prihvatljivim procentom identifikacije stražarskog limfnog čvora i niskom stopom lažno negativnih rezultata.

Ključne reči: karcinom dojke, biopsija, stražarski limfni čvor

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CASE REPORT

Cerebellar hemorrhage in full-term neonate: a case report and literature review

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Summary

Introduction/Objective: Cerebellar hemorrhage is a common condition in neonates born before the 32nd week of gestation, and it rarely occurs in full-term neonates. The most important risk factors for cerebellar hemorrhage in full-term neonates include traumatic delivery, instrumentation-assisted vaginal delivery, emergency cesarean section, perinatal asphyxia as well as perinatal infection.

Patient Review: We present a case of cerebellar hemorrhage and cerebral edema in a neonate with culture-negative early-onset sepsis. A full-term male neonate born from uncontrolled pregnancy developed respiratory distress, as well as clinical and laboratory signs of sepsis. The neonate's condition was complicated by respiratory failure, neurological deterioration and neonatal seizures. Chest radiography showed right-sided pneumonia and the head ultrasound showed cerebral edema and hemorrhage in right cerebellar hemisphere. Blood culture, tracheal aspirate and cerebrospinal fluid culture were sterile. The studies showed low incidence of blood culture confirmed early-onset sepsis due to high use of antibiotics in neonatal units. However, the course of the disease and resulting complications suggest that group B β-hemolytic Streptococcus may be a possible cause, as it is the most common pathogen responsible for early-onset sepsis in full-term neonates. Given the relationship between poor neurodevelopmental outcome in children and neonatal cerebellar hemorrhage, long-term follow-up by a pediatric neurologist is required.

Conclusion: Despite the incidence of cerebellar hemorrhage in full-term neonates is low, due to the poor neurodevelopmental outcome, head ultrasound through the "mastoid window" is advised in all critically ill neonates to detect cerebellar hemorrhage.

Keywords: cerebellar hemorrhage, brain edema, brain injury, newborn, sepsis

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INTRODUCTION

Cerebellar hemorrhage (CH) is the most common acquired lesion in the posterior cranial fossa (PCF) in the neonatal period, especially in preterm neonates born before the 32nd week of gestation. In full-term neonates, CH is a rare condition that affects critically ill neonates (1,2). The incidence of CH is inversely proportional to gestational age (GA) and birth weight (BW). The increase in the incidence of CH that has been reported in recent years due to advances in neuroradiological diagnostic techniques (3). In late preterm and full-term neonates, risk factors for CH include primiparity, traumatic delivery, assisted vaginal delivery using forceps and vacuum, occipital osteodiastasis, perinatal asphyxia, delivery completed by emergency cesarean section and the need for resuscitation in the delivery room, as well as perinatal infection. Also, coagulation disorders, such as fetal and neonatal alloimmune thrombocytopenia and vitamin K deficiency, and rare organic acidopathies can be the cause of CH (1). We present a full-term neonate with culture-negative sepsis complicated with pneumonia, cerebral edema (CE) and CH.

CASE PRESENTATION

A 40-week gestation, 2800 g male neonate was born via spontaneous vaginal delivery to a 30-year-old mother, this being her fourth uncontrolled pregnancy. The Apgar scores were 9 at one minute and 10 at five minutes after birth. The initial physical examination was normal in the delivery room. The neonate received 1 mg vitamin K and then was given to the mother for routine care in the Maternity Ward. At the end of the first day of life (DOL), the neonate developed signs of respiratory distress. His blood gas analyses showed respiratory acidosis, but complete blood count (CBC) showed thrombocytopenia and elevated concentration of C-reactive protein (CRP). Oxygen therapy was applied. After blood culture was taken, empirical antibiotic therapy (ampicillin and amikacin) with intravenous fluid infusion was started. In the further course, the neonate vomited fresh blood, so 1 mg of vitamin K was repeated and fresh frozen plasma was administered. The next day, meropenem was added to the therapy. On the third DOL there was a sudden clinical deterioration and the neonate was resuscitated, intubated, and placed on mechanical ventilation (MV).

The neonate was admitted to the Neonatal Intensive Care Unit (NICU) of the Institute of Neonatology on the third DOL in extremely severe condition, orotracheally intubated, in sopor, with generalized hypotonia, scarce spontaneous mobility and clinical signs of seizure with jerking movements of the limbs. The anterior fontanelle was not bulging. The seizure stopped after the neonate received loading dose of phenobarbital. After admission in our NICU, MV and antibiotic therapy were continued, as well as the maintenance dosage of phenobarbital. Additionally, due to hemodynamic instability, inotropic support by dopamine was started. Blood gas analysis showed a mild metabolic acidosis, and the CBC showed the leukocyte level of 31.500/mm^3 (neutrophils 65%, lymphocytes 19%, monocytes 3%), platelets of 44.000/mm^3, CRP of 59.5 mg/L. Chest radiography showed right-sided pneumonia. A head ultrasound (HUS) was performed, revealing signs of CE, including effacement of the cerebral sulci, compressed slit-like ventricles, and narrowing of the interhemispheric fissure **(Figure 1)**. Additionally, the scan showed hemorrhage in the right cerebellar hemisphere and increased echogenicity of the brain parenchyma.

Abdominal ultrasound and echocardiography showed normal findings. The next day the CBC showed a further increase in CRP of 98.2 mg/L, which was also the maximum value of CRP during hospitalization. In serum biochemistry analysis, the concentration of urea, creatinine, total protein, albumin and electrolyte, as well as activity of aspartate aminotransferase, alanine aminotransferase and

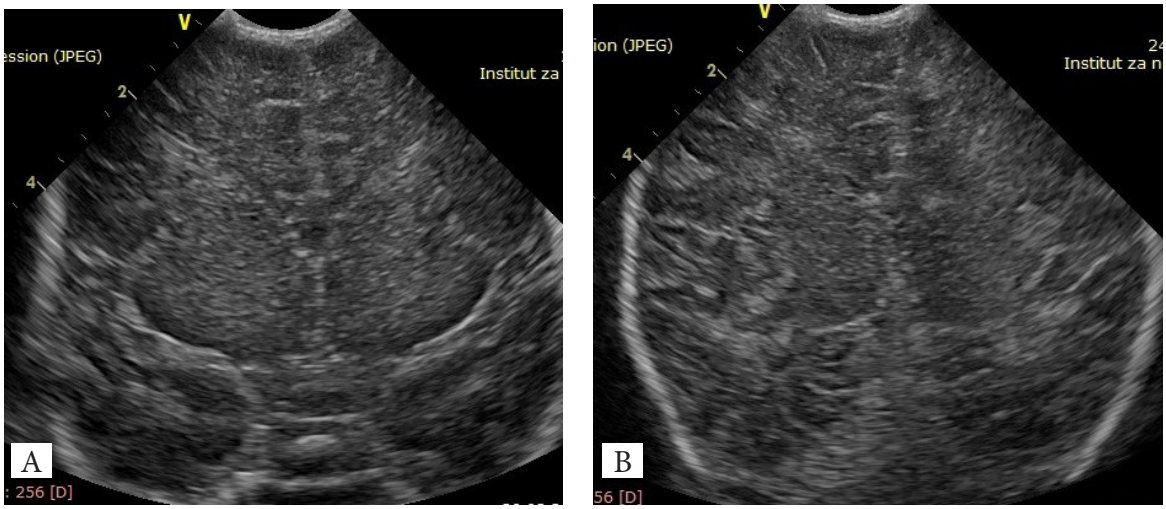

Figure 1. Head ultrasound showed effacement of the cerebral sulci, compressed slit-like ventricles, and narrowing of the interhemispheric fissure (A and B: coronal images)

γ-glutamyl transferase were within normal ranges. During the video electroencephalography (EEG), irregularity of the basic activity with epileptic discharges was recorded, so phenobarbital was continued. After extubation on the fourth day after admission, oxygen therapy was continued for 10 days. Cytological and biochemical analysis of cerebrospinal fluid (CBF) was normal. Blood culture, tracheal aspirate and CBF culture were sterile. During control HUS registered a gradual reduction of CE and CH. On control video EEG, the finding was normal for GA without epileptic discharges, the neonate had no seizures, so he was discharged home without phenobarbital.

DISCUSSION

This neonate admitted to the NICU due to early-onset sepsis (EOS) and neurological deterioration. Cerebral edema and CH associated with pneumonia and culture-negative sepsis were diagnosed in the neonate. In perinatal history, there were no common known risk factors for CH in full-term neonates such as perinatal asphyxia and hypoxic-ischemic encephalopathy (HIE), delivery by emergency cesarean section, resuscitation at birth, traumatic delivery and instrumentation-assisted vaginal delivery. The incidence of CH in full-term neonates is difficult to estimate because they undergo neuroimaging much less frequently compared to preterm neonates. In addition, small hemorrhagic lesions in the cerebellum are asymptomatic, so it is likely that these lesions often go undiagnosed (3-5).

Perinatal infection is also identified as a risk factor for cerebellar injury in the neonatal period (3,4). The retrospective study conducted in the NICU in Kuwait showed that the risk of CH in preterm neonates increased after infection with *Klebsiella pneumoniae* and *Enterococcus faecalis* (6). Similar studies in full-term neonates are rare, due to small sample sizes and predominantly single cases of CH. The cross-sectional study conducted in Canada identified 35% of mothers positive for group B β-hemolytic Streptococcus (GBS) at the time of delivery, whose neonates had cerebellar injury. However, there were no neonates with CH and non-hemorrhagic cerebellar lesions of positive blood culture for GBS (4). In our case, we had a sterile blood culture, tracheal aspirate and CBF culture, but the neonate had clinical and laboratory signs of sepsis. We had no data on maternal infections during pregnancy because it was uncontrolled. Also, due to high use of antibiotics in the NICU, the incidence of culture-positive EOS is low, about 0.4–0.8/1000 full-term neonates in high-income countries (7). In late preterm and full-term neonates, GBS and *Escherichia coli* are the leading causes of EOS and meningitis and are usually due to vertical transmission from a colonized mother (8). Complications of GBS meningitis, including arterial or venous cerebral infarction are described within the cerebellar hemisphere (9,10). Experimental studies have shown that GBS leads to changes in blood flow and perfusion within the brain parenchyma, as well as disrupting the integrity of the blood-brain barrier and overall impairing cerebral autoregulation. Additionally, GBS can cause damage to the endothelial cells of the brain's blood vessels (11-14). As a result of bacterial endotoxin activity, cytokines are released, triggering a systemic inflammatory response. During sepsis and septic shock, blood pressure instability, combined with impaired coagulation and/or thrombocytopenia, increases the risk of intracranial hemorrhage, including CH (6).

Besides sepsis and meningitis, several other complications of GBS infection in the neonatal period have been described, such as pneumonia and CE (15,16). These complications are also described in our case. However, despite sterile blood culture, tracheal aspirate and CBF culture, the clinical picture points to a high probability of GBS as the cause of sepsis and its complications. Other risk factors for CH in our case were MV, use of inotropic drug, and severe thrombocytopenia.

Full-term neonates with CH may present with irritability, apnea and seizures. These can also be signs of neonatal sepsis, so the clinical picture is non-specific, especially in minor CH. However, if these non-specific signs are associated with bulging fontanelle, separated sutures on the skull, bradycardia, horizontal deviation of the bulbs, facial paresis, intermittent tonic extension of limbs and opisthotonos, it is necessary to think about possibility of the brainstem compression. To confirm CH, it is necessary to perform HUS through the mastoid fontanelle **(Figure 2)**. This is especially true for critically ill neonates who have unexplained neurological signs, as well as signs of brainstem compression, and/or increased intracranial pressure (1,3). Although brain magnetic resonance imaging (MRI) is more sensitive neuroimaging modality, especially for the detection of pathological processes in the PCF, it is not the first method of choice in critically ill neonates (3,10). Our choice was HUS, because the neonate was not trans-

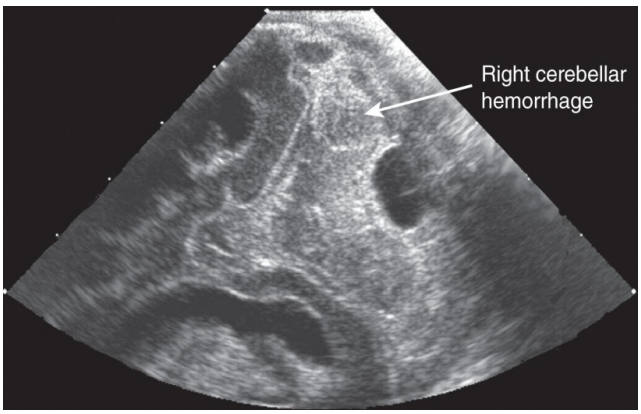

Figure 2. A head ultrasound of the posterior cranial fossa, obtained through the mastoid fontanelle, revealed a right cerebellar hemorrhage (from Limperopoulos C, Benson CB, Bassan H, et al. Cerebellar hemorrhage in the preterm infant: ultrasonographic findings and risk factors. *Pediatrics*. 2005; 116:717–724)

portable, and serial HUS examinations showed a gradual reduction of CH and CE with an improvement in the clinical findings of the neonate.

A limited number of studies have investigated the relationship between CH and neurodevelopmental outcome in full-term neonates. Infants and children with larger CH in neonatal period that led to cerebellar hemispheric and/or vermian atrophy had an increased risk for gross motor, cognitive, behavioral, and expressive language deficits (1,4). Therefore, in our case with CH and neonatal seizures at the time of discharge from the Institute, a brain MRI was advised, as well as a follow-up by a pediatric neurologist.

CONCLUSION

In conclusion, routine head ultrasound (HUS) examination through the anterior fontanelle, typically performed in preterm neonates, should also be conducted in full-term neonates, particularly those born from high-risk pregnancies. Although the incidence of CH in full-term neonates is low, due to the poor neurodevelopmental outcome, a HUS through the mastoid fontanelle is advised in all critically ill neonates to detect CH. It is necessary to carry out adequate prevention of intracranial hemorrhage, HIE and perinatal infection. Furthermore, additional studies are needed to explore cerebellar hemorrhage (CH) in full-term neonates and to investigate the relationship between perinatal infections and CH.

References

1. Limperopoulos C, du Plessis AJ, Volpe JJ. Cerebellar Hemorrhage. In: Volpe JJ, Inder TE, Darras BT, de Vries LS, du Plessis AJ, Ferriero DM, et al. editors. Volpe's Neurology of the Newborn. 7th ed. Philadelphia: Elsevier; 2024. p.760-776.
2. Pierson CR, Al Sufiani F. Preterm birth and cerebellar neuropathology. Semin Fetal Neonatal Med. 2016;21(5):305-311. https://doi.org/10.1016/j.siny.2016.04.006 PMID: 27161081.
3. Steggerda SJ, Meijler G. Neonatal cerebellar hemorrhage. UpToDate [Internet]. Available at: https://www.uptodate.com/contents/neonatal-cerebellar-hemorrhage (Accessed on 2024 July 4, 2024).
4. Limperopoulos C, Robertson RL, Sullivan NR, Bassan H, du Plessis AJ. Cerebellar injury in term infants: clinical characteristics, magnetic resonance imaging findings, and outcome. Pediatr Neurol. 2009;41(1):1-8. https://doi.org/10.1016/j.pediatrneurol.2009.02.007 PMID: 19520266.
5. Steggerda SJ, de Bruïne FT, Smits-Wintjens VE, Verbon P, Walther FJ, van Wezel-Meijler G. Posterior fossa abnormalities in high-risk term infants: comparison of ultrasound and MRI. Eur Radiol. 2015;25(9):2575-2583. https://doi.org/10.1055/s-0033-1347349 PMID: 25899415
6. Kartam M, Embaireeg A, Albalool S, Almesafer A, Hammoud M, Al-Hathal M, et al. Late-onset Sepsis in Preterm Neonates is Associated with Higher Risks of Cerebellar Hemorrhage and Lower Motor Scores at Three Years of Age. Oman Med J. 2022;37(2):e368. https://doi.org/10.5001/omj.2022.41 PMID: 35441041
7. Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. Front Pediatr. 2018; 6:285. doi: 10.3389/fped.2018.00285.
8. Palić I, Djordjević-Vujičić A, Palić D. Newer Insights in Etiology and Pathogenesis of Neonatal Sepsis. SVOA Paediatrics. 2024,3(4):79-88. http://doi.org/10.58624/SVOAPD.2024.03.070
9. Steggerda SJ, de Bruïne FT, Smits-Wintjens VE, Walther FJ, van Wezel-Meijler G. Ultrasound detection of posterior fossa abnormalities in full-term neonates. Early Hum Dev. 2012;88(4):233-239. https://doi.org/10.1016/j.earlhumdev.2011.08.011 PMID: 21924565
10. Fumagalli M, Parodi A, Ramenghi L, Limperopoulos C, Steggerda S. Ultrasound of acquired posterior fossa abnormalities in the newborn. Pediatr Res. 2020;87(Suppl 1):25-36. https://doi.org/10.1038/s41390-020-0778-9 PMID: 32218537
11. Zhang Q, Zhao M, Jiang W, Chen C. Intracranial hemorrhage associated with late-onset group B *streptococcus disease-a case report and a review of literature.* Transl Pediatr. 2020;9(1):61-65. http://dx.doi.org/10.21037/tp.202 PMID: 32154136
12. Tavares T, Pinho L, Bonifácio Andrade E. Group B Streptococcal Neonatal Meningitis. Clin Microbiol Rev. 2022;35(2):e0007921. http://doi.org/10.1128/cmr.00079-21 PMID: 35170986
13. Hauck W, Samlalsingh-Parker J, Glibetic M, Ricard G, Beaudoin MC, Noya FJ, et al. Deregulation of cyclooxygenase and nitric oxide synthase gene expression in the inflammatory cascade triggered by experimental group B streptococcal meningitis in the new-born brain and cerebral microvessels. Semin Perinatol. 1999;23(3):250-260. https://doi.org/10.1016/S0146-0005(99)80070-6 PMID: 10405195
14. Rudinsky BF, Lozon M, Bell A, Hipps R, Meadow WL. Group B streptococcal sepsis impairs cerebral vascular reactivity to acute hypercarbia in piglets. Pediatr Res. 1996;39(1):55-63. https://doi.org/10.1203/00006450-199601000-00008 PMID: 8825386
15. Morgan K, Baca N. Late-Onset Group B Streptococcal Meningitis in Infants. Pediatr Neurol. 2015;53(2):175-176. http://doi.org/10.1016/j.pediatrneurol.2015.04.015
16. Takahara T, Matsubara K, Maihara T, Yamagami Y, Chang B. Ultra-late-onset Meningitis Caused by Serotype IX Group B Streptococcus. Pediatr Infect Dis J. 2015;34(7):801. http://doi.org/10.1097/INF.0000000000000732 PMID: 26065666

CEREBELARNO KRVARENJE KOD TERMINSKOG NOVOROĐENČETA: PRIKAZ SLUČAJA I PREGLED LITERATURE

Ilija Palić[1], Katarina Kostić[1], Stevan Vasiljević[2]

Sažetak

Uvod: Cerebelarna hemoragija je često stanje kod novorođenčadi rođene pre 32. nedelje gestacije i viđa se retko kod terminske novorođenčadi. Najvažniji faktori rizika za cerebelarnu hemoragiju kod terminske novorođenčadi su traumatski porođaj, instrumentalno završen vaginalni porođaj, hitan carski rez, perinatalna asfiksija, kao i perinatalna infekcija.

Opis pacijenta: Predstavljamo slučaj cerebelarne hemoragije i edema mozga kod novorođenčeta sa sepsom sa ranim početkom i sterilnom hemokulturom. Terminsko novorođenče, rođeno iz nekontrolisane trudnoće, razvilo je respiratorni distres, kao i kliničke i laboratorijske znake sepse. Stanje novorođenčeta se komplikovalo respiratornom insuficijencijom, pogoršanjem neurološkog statusa i neonatalnim konvulzijama. Radiografija grudnog koša pokazala je desnostranu pneumoniju, a ultrazvuk glave edem mozga i hemoragiju u desnoj cerebelarnoj hemisferi. Hemokultura, trahealni aspirat i cerebrospinalna tečnost su bili sterilni. Studije su pokazale nisku incidencu hemokulturom potvrđenu sepsu sa ranim početkom usled visoke upotrebe antibiotika u neonatalnim jedinicima. Međutim, tok bolesti i nastale komplikacije sugerišu kao mogući uzročnik β-hemolytic Streptococcus grupe B, kao najčešći uzročnik sepse sa ranim početkom kod terminske novorođenčadi. S obzirom na povezanost lošeg neurorazvojnog ishoda kod dece i neonatalne cerebelarne hemoragije, potrebno je dugoročno praćenje od strane dečjeg neurologa.

Zaključak: Uprkos tome što je incidenca cerebelarne hemoragije kod terminske novorođenčadi niska, usled lošeg neurorazvojnog ishoda, ultrazvuk glave kroz "mastoidni prozor" je preporučen kod sve kritično bolesne novorođenčadi u cilju detekcije cerebelarne hemoragije.

Ključne reči: cerebelarna hemoragija, edem mozga, oštećenje mozga, novorođenče, sepsa

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