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More Than Fifty Years of Excellence - and Looking Ahead

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

Dear Readers,

It is my great pleasure to welcome you to the *Medical Research Journal*, a peer-reviewed scientific journal published by the Faculty of Medicine, University of Belgrade. Established in 1971, the journal has proudly maintained a tradition of academic excellence for over five decades.

Published four times annually, the *Medical Research Journal* is committed to advancing biomedical science by publishing high-quality research from both members and affiliates of the Belgrade Faculty of Medicine, as well as from scientists and professionals engaged in biomedical research worldwide. Our mission is to serve as a platform for the dissemination of innovative and impactful research that contributes to the development of medical science and clinical practice.

The journal publishes original scientific articles, review papers, case reports, and other types of contributions submitted in English. We welcome submissions that present previously unpublished results of original research, offer critical and comprehensive analyses of current topics, or describe unique and particularly illustrative clinical cases. Our journal encompasses research in basic biomedical sciences, clinical medicine, and preventive healthcare. Each manuscript undergoes statistical review to ensure methodological and analytical rigor. In line with our open-access policy, all published content is freely available to the public, supporting transparency, accessibility, and the global exchange of knowledge.

Looking ahead, our goal is to further elevate the journal's visibility and academic influence. We are fully committed to transitioning from our current national categorization (M53) to recognition as an international journal (M23). We are actively pursuing inclusion in prominent international indexing databases, aiming to increase the visibility and impact of the research we publish. Our long - term vision includes strategic efforts to enhance the quality, diversity, and global reach of our submissions, with a focus on fostering international collaboration and multidisciplinary research. While this journey is demanding, it is essential—and entirely possible through the joint commitment of our editorial team, authors, reviewers, and readers.

On behalf of the entire Editorial Board, I invite you to explore the latest contributions in this issue, and I warmly encourage researchers to consider the *Medical Research Journal* as a venue for publishing their work. Together, we will continue to promote excellence in medical science.

With sincere appreciation,
Prof. Dragana Protic, MD, PhD
Editor-in-Chief

ORIGINAL ARTICLE

Treatment of patients with acute promyelocytic leukemia using AIDA regiments: 20-year single-center experience

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Summary

Introduction: The introduction of all-trans retinoic acid (ATRA) has transformed acute promylocitic leucaemia (APL) from a highly fatal to a curable disease. However, significant frequency of early death (ED) and relapse represent challenges in everyday practice. The aims of this study are to report our 20 years long experience using AIDA-based protocols and to assess the predictive value of clinical and laboratory parameters for ED/relapse development.

Material and methods: This retrospective study included patients treated at the Clinic for Hematology, UKCS in the period 2004-2024. RS was defined as death within the first 30 days of hospitalization.

Results: 158 patients were included. ED and relapse occurred in 38/158 (24%) and 15/119 (12.6%) patients. ED rate stayed stable through time (2004-2008 period 20.6%, 2009–2013 21.9%, 2014–2018 23.1%, 2019–2024 27%) with bleeding as the most frequent cause (42%), followed by DS (24%) and infection (16%). Final predictors for ED development were ISTH DIC score (p=0.008, OR 2.38, 95% *CI* 1.25-4.53), aPTT (p=0.009, OR 0.79, 95% *CI* 0.66-0.94), ECOG PS (p<0.001, OR 2.96, 95% *CI* 1.63-5.39). Predictors for relapse were: ECOG PS ≥ 3 (21.7% vs 78.3%, p=0.019) and bcr3 PML-RARA transcript (29.6% vs 70.4%, p=0.046).

Conclusion: Our experience showed a very high rate of ED, with haemorrhage, DS and infection as a main reason. Preventive strategies should include comprehensive medical education, regarding prompt recognition, appropriate early transfusion support therapy, and the rapid initiation of ATRA. Patients with high ECOG PS and ISTH DIC score probably need different coagulation monitoring and reinvented therapy.

Keywords: acute promyelocytic leukemia, early death, relapse

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INTRODUCTION

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML), representing 10–15% of newly diagnosed AML cases (1–4). APL is cytogenetically characterized by the presence of a balanced translocation involving the retinoic acid receptor alpha (*RARA*) gene on chromosome 17 and promyelocytic leukemia (*PML*) gene on chromosome 15, resulting in the formation of the *PML: RARA* fusion gene, the driver gene for APL development (2-4). The clinical presentation of APL is aggressive, frequently marked by disseminated intravascular coagulation (DIC), severe bleeding and hemorrhagic death, which framed APL as the most lethal AML (2–4). However, following the introduction of all-trans retinoic acid (ATRA) and subsequently arsenic trioxide (ATO) APL has become acute leukemia with the best therapeutic responses (2–5). Different study groups reported early death (ED) rate between 5 -10% and relapse rate (RR) up to 12% resulting in complete remission (CR) rate as > 90%, 10-year survival of > 80% (2–5). However, highly selected patients in clinical studies probably do not represent the real-world population. Different APL registries, database as well as single center experiences reported significantly higher ED rate, ranging between 20-30% (5–11). On the other hand, RR is stable in all reports with a sharp decrease after the introduction of chemotherapy free, ATO + ATRA regiments (2–11). Therefore, reducing ED rate is a top priority in the treatment of APL. Several predictive factors for ED, such as age, socioeconomic status (SES), marital status, poor performance status (PS), high white blood cell (WBC) and peripheral blast counts, elevated serum lactate dehydrogenase (LDH), low fibrinogen level, low platelet count, prolonged prothrombin time (PT), high International Society on Thrombosis and Hemostasis (ISTH) DIC score were identified as predictive (2–11). Additionally, a few predictive models were constructed (2–11). However, there is not a generally recommended preemptive approach for reducing ED (8, 9).

The aim of our single-center retrospective study is to report our 20-year experience using AIDA based, PETHEMA protocols in the treatment of patients with APL. Moreover, we tried to assess the potential predictive value of some clinical and laboratory parameters for ED and relapse development.

MATERIAL AND METHODS

Patients and APL diagnostic procedures

Our study included 158 consecutive APL patients who were diagnosed and treated at the Clinic of Hematology, University Clinical Center of Serbia, from 2004 to 2024. The diagnosis was made using morphology, cytogenetics, molecular genetics and immunophenotyping of bone marrow (BM). The morphological diagnosis was made according to the contemporary World Health Organization (WHO) classifications of myeloid neoplasms and acute leukemia (12, 13). Cytogenetic analysis was performed by using conventional G-band karyotyping (14, 15). In all patient's detection *PML::RARA* rearrangements were performed by using reverse transcriptase polymerase chain reaction (RT-PCR) (15). Immunophenotyping was performed at diagnosis by direct multicolor immunofluorescence with a wide panel of monoclonal antibodies (CD2, CD3, cCD3, CD7, CD11a, CD11b, CD11c, CD13, CD15, CD16, CD19, CD33, CD34, CD38, CD45, CD56, CD64, CD114, CD117, HLA-DR, MPO) applied to the whole bone marrow specimen (16). Positivity was defined as expression in ≥ 20% of cells.

Clinical and treatment data

All patients were treated with AIDA regimens, including induction cycle of ATRA plus idarubicin, followed by three ATRA plus polychemotherapy consolidation cycles (17-19). In the period from September 2004 to August 2010 we used PETHEMA APL 99, from September 2010 to May 2013 PETHEMA APL 2005, and since June 2013 we have been using PETHEMA APL 2012 protocol (17-19). Differentiation syndrome (DS) prophylaxis applies in patients with initial WBC > $5x10^9$/L with dexamethasone from day 1 to day 15 (17-21). DS was diagnosed if ≥2 of the following signs were present: dyspnea, unexplained fever, weight gain ≥ 5 kg, unexplained hypotension, acute renal failure, and chest radiograph demonstrating pulmonary infiltrates or pleuro-pericardial effusion (17-21). Patients with confirmed DS were treated with therapeutic dexamethasone dose and in some cases ATRA was withheld until the patient's condition stabilized (17-21). Death from any causes that occurred during the initial 30 days after hospital admission were defined as ED (22-23). Bleeding events were graded according to the modified WHO grading system (24-25). Bleeding associated with moderate or severe hemodynamic instability, requiring red blood cell transfusion or central nervous system (CNS) bleeding, were classified as severe. Transfusions of platelet concentrates were used to obtain platelet count > 30-50 $\times10^9$/L, while cryoprecipitate was used to obtain fibrinogen level > 1-1.5 g/L. Fresh frozen plasma was transfused in case of INR > 1.5. These supportive measures were applied until the correction of coagulopathy, previously defined as normalization of fibrinogen and PT and bleeding cessation (17-19, 21). All patients were followed up from the diagnosis to death or the final check-up.

The retrieval of information and publication of these results were approved by the Institutional Review Board of the University Clinical Center of Serbia (protocol number 1435/10, 11-102-102, date: September 08, 2011). Patient consent was waived due to retrospective nature of study.

Data collection

We collected the following data: age, gender, bleeding at presentation, Eastern Cooperative Oncology Group performance status (ECOG PS), complete blood count, fibrinogen, PT, activated partial thromboplastin time (aPTT), D-dimer, immunophenotype, cytogenetics and molecular genetics, presence of DS during hospitalization. The Sanz risk score was used to classify patients to low (WBC ≤10x10^9/L, platelet count ≥ 40x10^9/L) intermediate (WBC ≤10x10^9/L, platelet count ≤ 40x10^9/L), and high-risk group (WBC > 10x10^9/L). The DIC diagnosis was made according to the recommendations of the International Society on Thrombosis and Hemostasis (ISTH). Patients with ISTH DIC score ≥ 5 were considered to have overt DIC (17, 18).

Statistical Analyses

Statistical analysis was performed using IBM SPSS statistical software (SPSS for Windows, release 25.0, SPSS, Chicago, IL, USA). We used absolute or relative frequencies to present categorical variables, which were compared using either Chi-square test or the Fisher's exact test. The Kolmogorov-Smirnov test and histogram were used to assess the normality of data distribution. Continuous variables with normal distribution are shown as mean and standard deviation (mean ± SD), while variables not complying with normal distribution are presented as median and range. T-test or Mann-Whitney U test were used for comparisons between groups for continuous variables. The significance level was set at 0.05. Univariate and multivariate logistic regression analyses were used to calculate and validate the risk factors for ED development. Variables with a $p < 0.05$ in the univariate model were included in the multivariate logistic regression analysis.

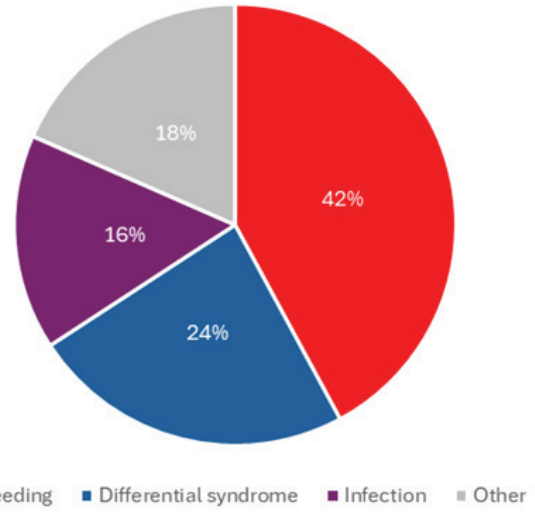


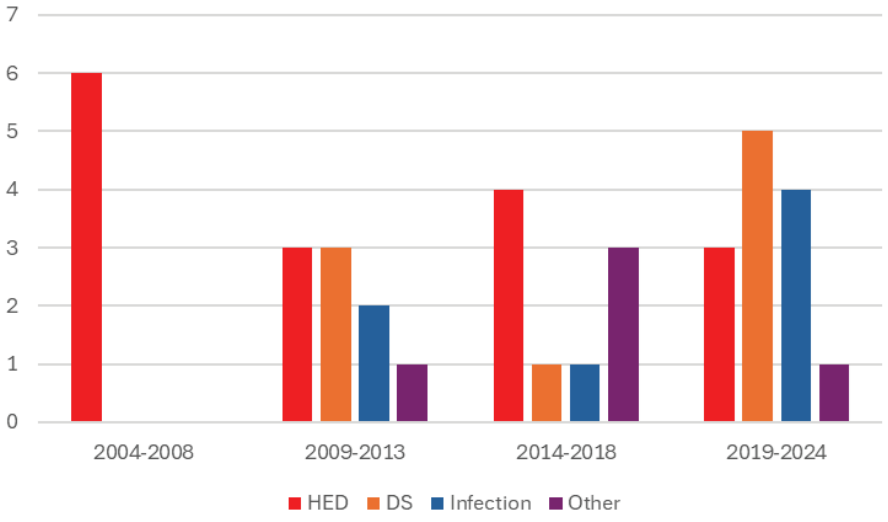
Figure 1. Causes of ED among our group of patients

Results

Among 158 patients of median age 45.3 years (range 18-78), 81 (53.8%) were females. At presentation, mild hemorrhagic syndrome was noted in 126 (85.7%) patients, severe hemorrhage in 9 (9.1%) patients, while bleeding was absent in 12 (10.9%) patients. Diagnosis of DS was made in 44/158 (27.8%) subjects. Early death occurred in 38/158 (24%). Bleeding was the most frequent cause of ED, followed by DS and infection **(Figure 1)**.

CR after induction cycle was achieved in 119/120 (99.2%) of patients. Consolidation death rate was 5/120 (4.2%). All patients died due to infections. RR was 15/119 (12.6%). Median overall survival was not reached.

To analyze trends in early death (ED) rates over time, the 20-year period was divided into four 5-year intervals. In the first period 2004 – 2008 ED rate was 6/29 (20.6%), in the second (2009 – 2013) ED rate was 9/41 (21.9%), in the third (2014 – 2018) it was 9/39 (23.1%) and in the last one (2019 – 2024) 15/39 (27). The distribution of early deceased patients by cause of death is shown in **Figure 2**.



Abbreviations: HED – hemorrhagic early death, DS - Differential syndrome

Figure 2. Distribution of ED causes overtime

Early death predictors

The clinical and laboratory features and comparison of all APL patients are shown in **Table 1**. Patients who experienced ED were significantly older (50.4 vs. 43.7 years, $p = 0.023$), had a higher ECOG performance status (ECOG PS ≥ 3 in 81.6% vs. 35.8%, $p < 0.001$), and more frequently presented with severe bleeding (16.2% vs. 2.7%, $p = 0.001$). They also had higher WBC counts (16.2×10^9/L vs. 2.6×10^9/L, $p = 0.001$), a higher incidence of high Sanz risk score (57.9% vs. 26.7%, $p < 0.001$), elevated D-dimer levels (31.0 vs. 18.8, $p = 0.002$), prolonged PT (57.8% vs. 7.0%, $p < 0.001$), shorter aPTT (25.4 s vs. 26.9 s, $p = 0.016$), and higher ISTH DIC scores (6 vs. 5, $p < 0.001$). Additionally, variant disease type was more common in this group (18.4% vs. 5.0%, $p = 0.016$), as was the development of differentiation syndrome (DS: 52.9% vs. 22.8%, $p = 0.001$).

Due to the multicollinearity between Sanz risk score and WBC as well as platelet count, two last variables were omitted from the model. Moreover, due to multicollinearity between ISTH DIC and platelet count, PT and D-dimer, only ISTH DIC score were included in the model. Given the number of identified predictors relative to the limited number of outcomes, we opted to perform logistic regression in two steps. In the first step, the predictors are divided into two groups. In the second step, variables that were significant in the initial two models were included in the final model.

Multivariant model 1 included patient and disease characteristics **(Table 2)**. Significant predictors for ED were: ECOG PS ($p < 0.001$, *OR* 2.73, 95% *CI*: 1.69-4.40), DS development ($p = 0.008$, *OR* 3.65, 1.40-9.47) and variant disease type ($p = 0.028$, *OR* 5.14, 1.20-22.07).

Table 2. Multivariate model 1

Step 1	B	p	0R	95% *CI*
Age	0.028	0.080	10.28	0.99-1.06
ECOG	1.003	< 0.001	2.73	1.69-4.40
DS	1.294	0.008	3.65	1.40-9.47
Variant disease type	1.637	0.028	5.14	1.20-22.07

Abbreviations: ECOG PS - Eastern Cooperative Oncology Group Performance Status, DS - Differential syndrome.

Multivariant model 2 included laboratory parameters **(Table 3)**. Significant predictors of ED were bleeding on admission ($p = 0.048$, *OR* 5.34, 1.01-28.18), ISTH DIC score ($p = 0.001$, *OR* 2.58, 1.45-4.60) and aPTT ($p = 0.033$, *OR* 0.86, 0.74-0.99)

Table 1. Comparison of clinical and laboratory data between alive and early deceased patients

		All (n = 158)	ED patients (n = 38)	Alive (n = 120)	P
Female, n (%)		85 (53.8)	22 (57.9)	63 (52.5)	0.561
Age, mean±sd		45.3±15.9	50.4±14.0	43.7±16.2	**0.023**
ECOG PS, n (%)	0	15 (9.5)	0 (0.0)	15 (12.5)	**< 0.001**
	1	66 (41.8)	7 (18.4)	59 (49.2)	
	2	37 (23.4)	11 (28.9)	26 (21.7)	
	3	31 (19.6)	14 (36.8)	17 (14.2)	
	4	9 (5.7)	6 (15.8)	3 (2.5)	
	5	0 (0.0)	0 (0.0)	0 (0.0)	
Bleeding on presentation, n (%)	Without	12 (8.2)	0 (0.0)	12 (10.9)	**0.001**
	Moderate	126 (85.7)	31 (83.8)	95 (86.4)	
	Severe	9 (9.1)	6 (16.2)	3 (2.7)	
Hb (g/L), mean±sd		96.9±21.2	92.0±19.6	98.4±21.6	0.106
WBC (x109/L), median (range)		3.6 (0.4-208.8)	16.2 (0.4-208.8)	2.6 (0.4-91)	**0.001**
Plt (x109/L), median (range)		27.0 (0-279)	26 (0-64)	30 (2-279)	0.066
Sanz high risk, n (%)		54 (34,2)	22 (57.9)	32 (26.7)	**< 0.001**
Fibrinogen (g/L), median (range)		2.7 (0.4-8.8)	2.27 (0.9-8.8)	2.8 (0.4-6.2)	0.210
PT (%), mean±sd		67,0±17.0	57.8±16.5	69.9±18.8	**< 0.001**
aPTT (s), mean±sd		26.5±4.0	25.4±2.8	26.9±4.2	**0.016**
D dimer (μg/L), median (range)		24.0 (0.70 – 2345.0)	31.0 (2.1-233.0)	18.8 (0.7-2345.0)	**0.002**
ISTH DIC, median (range)		6 (1-8)	6 (5-7)	5 (1-8)	**< 0.001**
Variant disease type, n (%)		13 (8.3)	7 (18.4)	6 (5.0)	**0.016**
Additional cytogenetic abnormalities, n (%)		24 (15.8)	7 (19.4)	17 (14.7)	0.388
PML RARA, n (%)	Bcr 1	54 (60.7)	7 (63.6)	47 (60.3)	1.000
	Bcr 2	3 (3.4)	0	3 (3.8)	
	Bcr 3	32 (36.0)	4 (36.4)	28 (35.9)	
ATRA, n (%)		44 (29.7)	18 (52.9)	26 (22.8)	**0.001**

Abbreviations: ECOG PS -Eastern Cooperative Oncology Group Performance Status, Hb – hemoglobin, WBC – with blood cells, Plt – platelets, aPTT - activated partial thromboplastin time, ISTH DIC score - International Society on Thrombosis and Hemostasis disseminated intravascular coagulation score.

Table 3. Multivariate model 2

Variables	B	p	0R	95% *CI*
Sanz risk	0.180	0.628	1,20	0.58-2,47
ISTH DIC score	0.949	0.001	2.58	1.45-4.60
Bleeding	1.675	0.048	5,34	1.01-28.18
aPTT	-0.157	0.033	0,86	0.74-0.99

Abbreviations: aPTT - activated partial thromboplastin time

Our final model included: ECOG PS, DS development, variant disease type, bleeding on admission, ISTH DIC score and aPTT. Final predictors for ED development were ISTH DIC score (p = 0.008, *OR* 2.38, 1.25-4.53), aPTT (p = 0.009, *OR* 0.79, 0.66-0.94), ECOG PS (p < 0.001, *OR* 2.96, 1.63-5.39). Data are presented in **Table 4**.

Table 4. Final model

Variables	B	p	0R	95% *CI*
ISTH DIC score	0.867	0.008	2.38	1.25-4.53
Bleeding	1.142	0.293	3.13	0.37-26.39
aPTT	-0.241	0.009	0.79	0.66-0.94
ECOG PS	1.086	< 0.001	2.96	1.63-5.39
Variant disease type	0.991	0.198	2.69	0.60-12.16
DS	0.955	0.069	2.60	0.93-7.28

Abbreviations: ISTH DIC score - International Society on Thrombosis and Hemostasis disseminated intravascular coagulation score, aPTT - activated thromboplastin time, ECOG PS - Eastern Cooperative Oncology Group Performance Status, DS - Differential syndrome

Predictors of relapse

In the examined group of parameters, statistically significant difference showed ECOG PS ≥ 3 1(0/46, 21.7% vs 36/46, 78.3%, p = 0.019) and bcr3 PML RARA transcript (8/27, 29.6% vs 19/27, 70.4%, p = 0.046). All other parameters were not statistically significant different (female gender 8/62, 12.9% vs 54/62, 84.1%, p = 0.948); age (51.33 vs 60.69), p = 0.322; variant disease type (1/6, 16.7% vs 5/6, 83.3%, p = 0.569); additional cytogenetic abnormalities (4/16, 25% vs 12/16, 75%, p = 0.213), Hb level (101.86 vs 96.76, p = 0.357); WBC (4.85 vs 2.4, p = 0.235); platelet count (28.50 vs 29/00, p = 0.810), Sanz risk score (high risk 6/32. 18,8% vs 9/86, 10,5% p=0.231), fibrinogen (2.24 vs 2.92, p = 0.094), PT (66.79% vs 69.09%, p = 0.787), aPTT (27.84 vs 26.48, p = 0.331), D dimer (132.65 vs 223.6, p = 0.944), ISTH DIC score > 4 (2/27, 7.4% vs 12785, 14.1/, p = 0.512), Differential syndrome development (9/88, 10.2% vs 79/88, 89.2%, p = 0.299).

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Clinical Center (protocol number 1435/10, 11-102-102, date 08.09.2011.). Informed consent was obtained from all subjects involved in the study after September 8th, 2011. Due to the retrospective nature of the study, the inform consents were waived before this date.

DISCUSSION

The introduction of ATRA and ATO have transformed APL from a highly fatal to a highly curable disease (2-11). However, significant frequency of ED and occurrence of relapse remain a challenge in everyday clinical practice.

In our group of patients, the ED rate was 24%, which is significantly higher in comparison to reports from clinical studies, but in line with data from registries, national or single center experience publications (2-11). ED rate has often been significantly underestimated in clinical studies due to exclusion of patients with high ECOG PS, comorbidities and even more patients who died even before the treatment (8, 9).

Over decades, the frequency of ED in APL has been decreasing (7, 27). With the widespread introduction of ATRA into clinical practice after 1995, the ED rate was significantly reduced for the first time. A subsequent decline was observed after 2006, coinciding with advancements in the medical environment, including the implementation of aggressive rescue measures, improved supportive care, and better management of complications. However, after these two periods the ED rate has remained stable (7, 27). In our group of patients treated since 2004, a decrease in ED rate has not been observed. However, we observed a significant change in the rate of specific causes. HED remained a significant reason for ED, but the rate was reduced after the 2004-2008 period. This reduction is probably related to better transfusion support in our center. Moreover, since predictive value of conventional coagulation tests is not consistent, we recently investigated the utility of rotational thromboelastometry (ROTEM) in APL patients. According to our previous publication, ROTEM parameters could be surrogates for HED and beneficial in identifying at least those patients with excessive fibrinolysis who need different transfusion support (7, 28).

The frequency of deaths due to DS remained high in our cohort despite corticosteroid prophylaxis. However, the optimal prophylaxis scheme remains controversial (29). Additionally, patients with DS insensitive to steroids typically have a poor prognosis, highlighting the necessity for improved management strategies in this area. Early DS recognition as well as corticosteroid therapy initiation in full doses are essential (19, 29). Wu at al. suggested that inclusion of ruxolitinib for managing DS in APL resulted in a reduction of early death rate (19).

Unfortunately, during the last period we saw a significant increase in ED caused by infections. Currently, our infection-related mortality rate matches that reported by PETHEMA, likely due to improved diagnostic capabilities and better differentiation from pulmonary bleeding and DS. It is well documented that geographical distribution (high vs mild and low-income countries) make a significant impact on ED rate. Multidrug availability, considering antibiotics and antimycotics will significant-

ly reduce our infection-related ED rate (30-33). However, the availability of non-chemotherapy is more significant. Therapy based on ATRA and ATO combination is not myeloablative which results in a significant decrease of infection rate (6, 33, 34). New data from Harmony registry confirms the superiority of ATRA-ATO over ATRA-chemotherapy in patients with APL (6).

One of the strategies to reduce ED rate is to identify high risk patients. Factors that were previously identified as ED predictors such as age, WBC, low platelet count, Sanz score, prolonged PT, variant disease type were also identified as predictive in our univariate analyses but lost significance in the final model. After final multivariate analyses our model included ISTH DIC score, aPTT and ECOG PS. Patients with APL have: 2.38-time higher risk for ED with every point of ISTH DIC score rising; 0.786-time lower risk of ED with every second of aPTT prolongation and 2.964 higher risk of ED with every ECOG PS grade rising. Predictive value of disease-unrelated parameters such as ECOG PS probably can be explained by higher disease burden and represent sicker patients in which ED is more likely. Our data suggest that DIC is the major cause of bleeding and death in APL patients and that the degree of DIC may correlate with the risk of ED. Interestingly, a majority of studies with a focus on bleeding and HED did not assess predictive values of DIC scores (8). Patients with high risk probably need different therapy strategies. In the first step coagulation parameters monitoring should be intensified with a new global coagulation test adding to identified patients who may benefit from intensive transfusion support [8]. Although HED rate stays high with ATRA+ATO regiments, this approach will reduce ED rate associated with infections (5, 34, 35).

CONCLUSION

In conclusion, our 20-year long experience showed a very high rate of ED, with hemorrhage, DS and infection as main causes. Preventive strategies should include comprehensive medical education, especially in emergency departments, regarding prompt recognition, appropriate early transfusion support therapy, and the rapid initiation of ATRA (30-33). Using the ATO regiment can contribute to the further reduction of ED. However, patients with high ECOG PS and high ISTH DIC score probably need different coagulation and DS monitoring and reinvented therapy.

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Author contributions: M.M. researched literature, critically analyzed data and drafted the manuscript N.P., N.S. researched the literature, V.M. J.R., M.C., Z.P., LJ.J., V.Đ., V.K., T.D.I., S.B.R. collected data. Z.B., L.T. performed statistical analysis. N.S.V., A.B., A.V., N.K.K. and M.V. revised the manuscript. M.M., N.P., N.S., formulated the idea, analyzed the literature, and revised and critically appraised the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Clinical Center (protocol number 1435/10, 11-102-102, date: September 08, 2011). Informed consent was obtained from all subjects involved in the study after September 08, 2011. Due to the retrospective nature of the study, the inform consents were waived before this date.

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LEČENJE PACIJENATA SA AKUTNOM PROMIJELOCITNOM LEUKEMIJOM PRIMENOM AIDA PROTOKOLA: DVADESETOGODIŠNJE ISKUSTVO JEDNOG CENTRA

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Sažetak

Uvod: Uvođenje all-trans retinoične kiseline (ATRA) transformisalo je akutnu promijelocitnu leukemiju (APL) iz visoko smrtonosne u izlečivu bolest. Međutim, značajna učestalost rane smrti (RS) i relapsa i dalje predstavlja izazov u svakodnevnoj praksi. Ciljevi ovog rada su prikazati 20-godišnje iskustvo u lečenju bolesnika AIDA zasnovanim protokolima i proceniti prediktivnu vrednost kliničkih i laboratorijskih parametara za razvoj RS i relapsa bolesti.

Materijal i metode: U ovu retrospektivnu studiju su uključeni pacijenti lečeni u Klinici za hematologiju, UKCS u period 2004-2024. RS je definisana kao smrt u prvih 30 dana hospitalizacije.

Rezultati: U studiju je uključeno 58 pacijenata. RS i relaps su se javili kod 38/158 (24%) odnosno 15/119 (12,6%) pacijenata. Stopa RS je ostala stabilna tokom vremena (2004-2008 period 20,6%, 2009-2013 21,9%, 2014-2018 23,1%, 2019-2024 27%) sa krvarenjem (42%), diferencijacionim sindromom (DS, 24%) i infekcijama (16%) kao najčešćim uzročnicima. Konačni prediktori za razvoj RS bili su: ISTH DIC skor (p=0,008, *OR* 2,380, 95% *CI* 1,25-4,53), aPTT (p=0,009, *OR* 0,79, 95% *CI* 0,66-0,94), ECOG PS ($p < 0.001$, *OR* 2.96, 95% *CI* 1.63-5.39). Kao faktori povezani sa relapsom identifikovani su: ECOG PS ≥3 (21,7% naspram 78,3%, p=0,019) i bcr3 PML-RARA transkript (29,6% naspram 70,4%, p=0,046).

Zaključak: Veoma visoku stopa RS perzistira u našoj grupi, sa krvarenjem, DS i infekcijom kao glavnim uzrocima. Preventivne strategije bi podrazumevale sveobuhvatnu medicinsku edukaciju, sa ciljem brzog prepoznavanja, adekvatne terapije transfuzijama derivata i komponenata krvi, i brzom započinjanju terapije ATRA-om. Pacijentima sa visokim ECOG PS i ISTH DIC potrebno je intenzivnije praćenje koagulacionih paremetara i intenzivnija suportivna terapija.

Ključne reči: akutna promijelocitna leukemija, rana smrt, relaps

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ORIGINAL ARTICLE

Prolonged blockade of NMDA receptors and positive modulation of α5 $GABA_A$ receptors: no changes in depressive-like behavior, while the former slightly increased emotional reactivity in unstressed rats

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Summary

Introduction: Depression is a multifaceted disorder with a limited therapeutic repertoire. A significant breakthrough in depression research has been the discovery of fast-acting antidepressants that target the glutamate/GABA system, namely ketamine and neurosteroids. Positive modulation of $GABA_A$ receptors containing the α5 subunit (α5GABAARs) represents a promising new approach for targeted therapy of depression. The aim of this study was to investigate the effects of repeated administration of ketamine, an NMDA antagonist, and GL-II-73, a positive allosteric modulator at α5GABAARs, on behavioral despair, anxiety, and locomotor activity.

Material and Methods: The experiments were performed on adult male Sprague-Dawley rats. Animals were treated for 7 days with either 6 mg/kg or 10 mg/kg ketamine in an intermittent dosing regimen, 10 mg/kg GL-II-73, or solvent. Following treatment, we performed a battery of behavioral tests consisting of forced swim test (FST), spontaneous locomotor activity (SLA), and novelty suppressed feeding test (NSFT).

Results: No change was detected in any of the treatment groups regarding performance in the FST and NSFT. In SLA, all forms of treatment caused a decrease in the percentage of central time at both 10 and 30 minutes. Central distance was reduced at 10 and 30 minutes only after the 10 mg/kg dose of ketamine.

Conclusion: Ketamine at the higher dose (10 mg/kg) elicited behavioral changes consistent with a slight increase in emotional reactivity, while minor changes of the same quality in the lower-dose ketamine and GL-II-73 groups hardly reflect any anxiety-inducing influence.

Keywords: depression, anxiety, ketamine, α5 $GABA_A$ receptor modulation.

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INTRODUCTION

Depression is a multifaceted and widespread mental disorder that has a high prevalence worldwide and causes significant personal, social, and economic distress (1). Conventional treatments for depression, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), are slow-acting and take several weeks to achieve their full therapeutic effect (2). This delayed response, combined with the fact that a significant proportion of patients with major depressive disorder (MDD) experience inadequate symptom relief, highlights the urgent need for alternative therapies, particularly for treatment-resistant depression (TRD) (3). In this context, ketamine, a dissociative anaesthetic traditionally used in surgery, has emerged as a breakthrough intervention (4). Ketamine was initially studied for its anaesthetic properties. It was later found to have a rapid antidepressant effect when administered in subanesthetic doses (5). The mechanism of action is thought to be primarily related to N-methyl-D-aspartate receptor (NMDAR) antagonism, although alternative mechanisms have also been described (6). The beneficial effects are believed to be mediated by the ability to induce neuroplastic changes and improve neuronal connectivity, particularly in brain regions associated with mood regulation, including the prefrontal cortex and hippocampus (7).

Notably, the effects of ketamine on depression are not only rapid but also long-lasting, with some patients experiencing relief from depressive symptoms for up to a week or longer after a single infusion (8). This contrasts sharply with traditional antidepressants, which require prolonged use to elicit therapeutic effects. The potential for ketamine to offer rapid and sustained relief for individuals suffering from TRD has sparked significant interest and research in the field of psychiatric medicine (9). Studies have demonstrated that even in patients with severe, chronic depression who have not benefited from multiple rounds of other treatments, ketamine can produce significant improvements in mood, cognition, and overall functioning (9). In addition, there is evidence that ketamine may be effective in treating a range of depression subtypes, including patients with co-occurring anxiety and suicidality for whom other treatments have failed (10).

Despite its promising effect, the clinical use of ketamine in the treatment of depression is still fraught with problems. The optimal dosage, frequency of administration, and duration of effect are still under investigation (11). In addition, the safety profile of ketamine remains a concern, particularly with regard to its abuse potential, dissociative side effects, and long-term cognitive effects (12,13). The fact that ketamine produces psychedelic-like experiences in some individuals also raises questions about the psychological consequences of its use (14).

On the wave of the discovery of fast-acting antidepressants, innovative $GABA_A$ receptor (GABAAR) modulators have emerged as promising treatments for depression, particularly for patients who do not respond to traditional monoaminergic antidepressants (15). These modulators, such as brexanolone and zuranolone, target neurosteroid-sensitive GABAARs to enhance inhibitory neurotransmission and restore disrupted neural circuits implicated in depression (15). In contrast to conventional benzodiazepines, which predominantly potentiate synaptic GABAARs and thus enhance phasic inhibition, these novel modulators enhance both synaptic and extrasynaptic GABAAR function and thus potentiate phasic and tonic inhibition in parallel (16). Brexanolone, the first FDA-approved drug of this class, has demonstrated rapid antidepressant effects in postpartum depression, while zuranolone, an orally active analog, offers potential advantages in terms of accessibility and chronic use (17). By leveraging the role of GABAAR plasticity in stress-related disorders, these modulators represent a paradigm shift in antidepressant development, providing a mechanistically distinct and faster-acting alternative to traditional treatments (18).

GABAARs containing the α5 subunit (α5GABAARs) have garnered increasing attention in depression research due to their unique anatomical distribution and physiological role (19). Due to the ability of the α5 subunit to bind to key anchoring proteins located both synaptically and extrasynaptically, these receptors may mediate tonic inhibition in a regulated manner, which is critical for modulating network excitability and synaptic plasticity (19). Evidence suggests that augmenting α5GABAAR activity reverses age related neuronal atrophy and cognitive impairment (20). Preclinical studies indicate that selective positive allosteric modulators (PAMs) of α5GABAAR can exert antidepressant and procognitive effects and can reverse stress related neuronal pathologies (21-24). While there is open debate in the field about the apparent contradiction that ketamine and α5GABAAR PAMs induce antidepressant effects through differentially opposing mechanisms (15,25,26), a recent report indicating that time-dependent modulation of GABAergic activity is required for the sustained antidepressant-like responses induced by ketamine has shed new light on this topic (27).

Repeated administration of ketamine has been suggested to be more efficacious in inducing lasting antidepressant effects (28,29). However, ketamine use has been documented to cause structural brain changes (cortical atrophy) after chronic administration in drug abusers (30). Also, NMDAR antagonists such as phencyclidine and ketamine transiently induce symptoms of acute schizophrenia, and they have been reliably used to model schizophrenia features in rodents (31). Preclinical studies have drawn attention to the negative effects of chronic ketamine administration in rats, highlighting complex neurophysiological changes arising from the treatment (32). Having these facts in mind, it has been suggested that an intermittent dosing regimen of ketamine might

be a preferred treatment option in a protocol aimed to assess its antidepressant-like effects (32).

In this paper, we aimed to compare the behavioral effects of intermittent dosing of ketamine and repeated administration of GL-II-73, a widely used α5GABAAR PAM (21,23,24) in the domains of behavioral despair, anxiety, and locomotor activity in unstressed male rats.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats (n=40), descended from a colony purchased from Charles River, Italy, were born and reared in the vivarium of the Faculty of Pharmacy, University of Belgrade, Serbia. The vivarium environment was maintained at a temperature of 22 ± 2°C and a relative humidity of 40-70%. The animals received food and water ad libitum and were exposed to a 12/12-hour day/night light cycle, with the light being switched on at 06:00. The research was conducted in accordance with European Union guidelines (Directive 2010/63/EU). It was approved by the Ethics Committee for Animal Experiments of the University of Belgrade – Faculty of Pharmacy, Serbia and the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate (323-07-10046/2020-05, 23/09/2020).

Drug treatment

In this study, four groups of 10-12-week-old animals weighing approximately 300-350 g were subjected to different treatments administered by intraperitoneal injection. The first group received the solvent (SOL) consisting of 14% (w/v) propylene glycol and 1% (w/v) polysorbate 80 dissolved in ultrapure water, which served as a control. The second and third groups were administered ketamine hydrochloride (Ketamidor, Richter Pharma AG, Vienna, Austria) at a dosage of 6 mg/kg and 10 mg/kg, respectively, with the ketamine diluted in physiological saline to achieve the desired dosage. The fourth group received GL-II-73, the imidazobenzodiazepine derivative synthesized by the research group of Dr. James M. Cook (Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI, USA). The GL-II-73 solution was prepared in SOL at a concentration of 2 mg/ml, which corresponds to a final dose of 10 mg/kg. GL-II-73 is a PAM at the benzodiazepine binding site that is selective for α5GABAARs (24).

Experimental design

Before starting the protocol (day -1), the animals were placed individually in a cage and a 15-minute training session for the forced swim test was performed on this day. The treatment was applied for 7 days (0 - 6 days of the protocol). The control group received SOL i.p. daily, the Ket 6 and Ket 10 groups received ketamine i.p. on 0, 2, 4 and 6 days while receiving SOL i.p. on the other days, and the GL-II-73 group received GL-II-73 i.p. on all 7 days. This was followed by a series of behavioral tests: forced swim test (day 7), spontaneous locomotor activity (day 8), novelty suppressed feeding test and home-cage feeding (day 9). The experimental design is shown in **Figure 1**.

Behavioral testing

During the spontaneous locomotor activity (SLA) test, each experimental animal was allowed a 30-minute exploratory period in an open arena of specific dimensions (60 cm × 50 cm × 35 cm) illuminated with indirect bright light (60 lx). The animals were gently placed in the arena and their behavior was monitored using AnyMaze software version 6.35 (www.any-maze.com). The following parameters were tracked: the percentage of time the animals spent in the central zone during the 30-minute duration of the SLA test, as well as during the 10-minute exploration period; the total distance travelled and the percentage of active time during both the 30-minute and 10-minute durations of the SLA test (33).

The forced swim test (FST) was performed as previously described for rats. Each rat was pre-swum for 15 minutes in a Plexiglas cylinder (30 cm diameter, 65 cm height) filled with water (25 ± 1°C, 45 cm depth). After 8 days, each rat was again placed in the swimming cylinder for 10 minutes and videotaped. The data were analyzed in a blinded manner, and the total duration of immobility during the entire 10-minute swimming period was assessed. The duration of immobility was assessed between

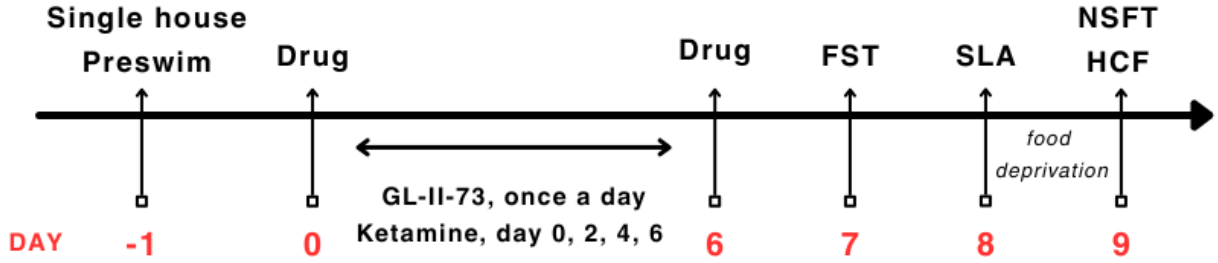

Figure 1. The schedule for conducting the experiment and the accompanying behavioral tests

2 and 6 minutes by an experimenter who was blind to the treatment groups. The parameters recorded included the time the animals spent in various activities during the 2-6 min period of the test: swimming, immobility, and struggling (34).

In the novelty suppressed feeding test (NSFT), the animals were deprived of food overnight and then placed in an arena with an open field (100 cm × 100 cm × 35 cm) and a small amount of food in the center. The latency time to the onset of food intake was recorded, with a maximum cut-off time of 15 minutes (34).

Following the NSFT, home-cage feeding (HCF) was assessed during a 10-minute period to determine the animals' motivation to eat (34).

Animal behavior was recorded with a camera and analyzed with AnyMaze software (Stoelting Co., Wood Dale, IL, USA).

Statistical analysis

Data were analyzed by Kruskal–Wallis test and Mann–Whitney test (GraphPad Prism software version 10.1.0; GraphPad Software, Inc., La Jolla, CA) with the Bonferroni-Holm correction for post hoc comparison (MultipleTesting.com) (35). A value of $p < 0.05$ was considered statistically significant. A technical outlier was observed in the SLA data due to a mistracking error in the AniMaze system.

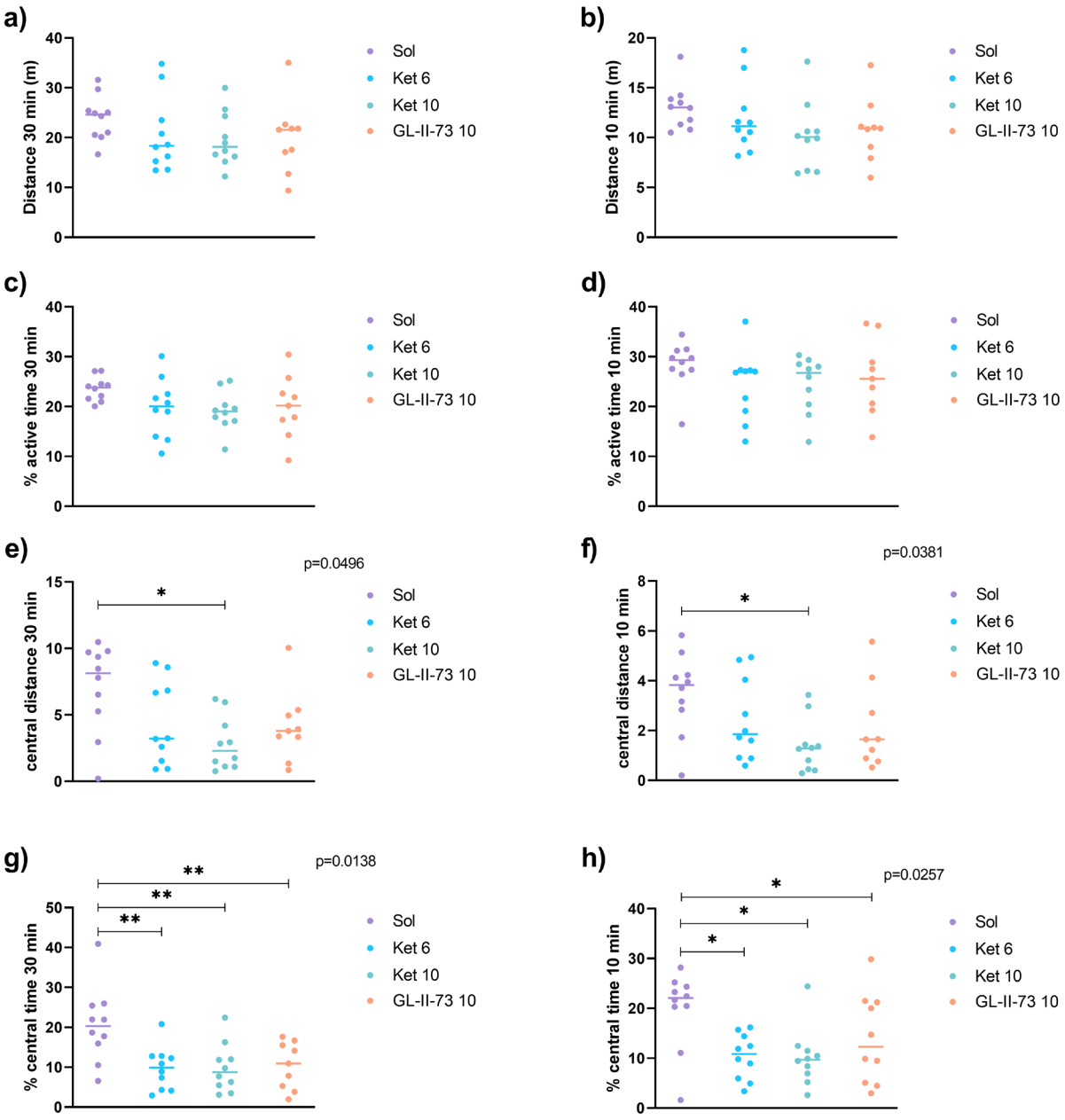

Figure 2. Assessment of locomotor activity and anxiety in male Sprague-Dawley rats in the spontaneous locomotor activity test. The total distance travelled in 30 minutes (a) and in 10 minutes (b), the percentage of active time in 30 minutes (c) and in 10 minutes (d), the central distance travelled in 30 minutes (e) and in 10 minutes (f), the percentage of time spent in the central zone in 30 minutes (g) and in 10 minutes (h) are shown 24 hours after the last application. Statistical significance is indicated in the graphs with * for $0.01 < p < 0.05$, ** for $0.001 < p < 0.01$.

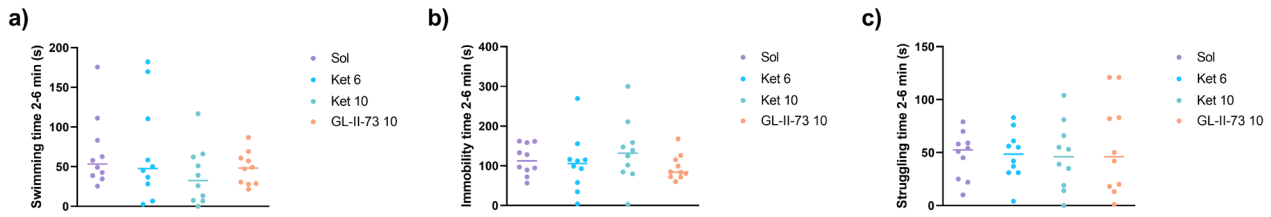

Figure 3. Results of the forced swim test. The data presented show the time the animals spent on different activities during the 2 and 6 minutes of the test: swimming (a), immobility (b) and struggling (c).

RESULTS

Spontaneous locomotor activity (SLA) test

The total distance travelled and the percentage of active time in 30 minutes **(Figure 2a, 2c)** as well as the total distance travelled and the percentage of active time in 10 minutes in the SLA test **(Figure 2b, 2d)** did not differ significantly in any of the groups.

A significant decrease in the central distance parameter was observed during both the 30 minutes **(Figure 2e)** and the 10 minutes **(Figure 2f)** of the test in the Ket 10 group compared to the control group of animals (p=0.007, respectively).

A significant reduction in the percentage of time the animals spent in the central zone during the 30-minute duration of the SLA test **(Figure 2g)** was observed in all three treatment groups, Ket 6, Ket 10, and GL-II-73 (p=0.007; p=0.009; p=0.008, respectively) compared to the control group. In addition, a significant decrease in the percentage of time the animals spent in the central zone during the 10-minute duration of the SLA test was observed in the three treatment groups (p=0.007; p=0.018; p=0.022, respectively) compared to the control group **(Figure 2h)**.

Forced swim test (FST)

There is no significant difference in any of the groups in swimming **(Figure 3a)**, immobility **(Figure 3b)** and struggling **(Figure 3c)** in FST.

Novelty suppressed feeding test (NSFT) and home-cage feeding (HCF)

There is no significant difference in any of the groups in the latency time to food intake in NSFT **(Figure 4a)** and food intake in HCF **(Figure 4b)**.

DISCUSSION

Behavioral studies that ensued 24 hours after the last administration of ketamine at a dose of 6 mg/kg or 10 mg/kg or GL-II-73 at a dose of 10 mg/kg showed that none of the treatments significantly decreased immobility time in the FST or latency to food intake in the NSFT, which were the previously demonstrated effects of 10 mg/kg ketamine (34). Analysis of SLA behavior showed that activity in the central parts of the chamber seemed to be differentially reduced in animals previously subjected to the treatments studied. Ketamine at doses of 6 and 10 mg/kg, as well as 10 mg/kg GL-II-7 decreased the percentage of central time at both 10 and 30 minutes. The central distance was reduced at 10 and 30 minutes only after the 10 mg/kg dose of ketamine.

When manifested in the central parts of an activity chamber, the decrease in locomotor activity may reflect an increase in emotional reactivity, whereas when manifested in the peripheral parts of a cage, such locomotor changes may rather reflect a vague "lack of well-being" (36) or a decrease in exploratory drive in general (cf. 37). On the other hand, the emotional reactivity (related to neophobia) disappears once the animal has become ha-

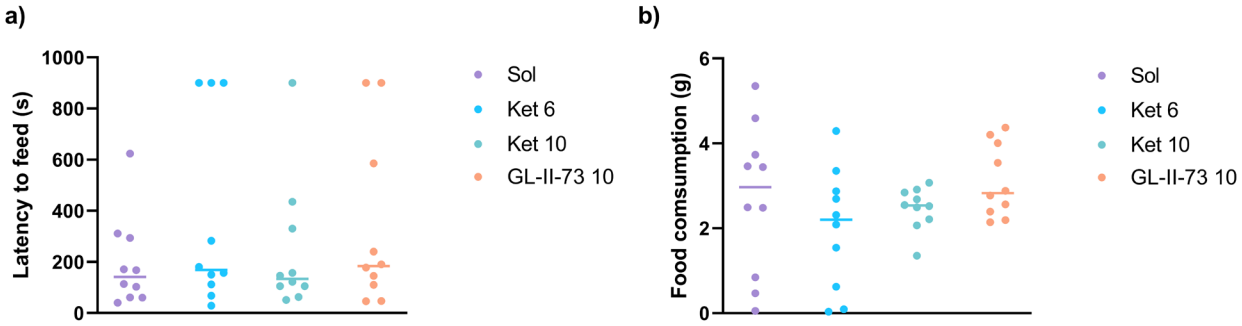

Figure 4. Results of a novelty suppressed feeding test and home-cage feeding. The data represented the time it took an animal to start eating (a) and the amount of food it consumed within 10 minutes in its home cage (b).

bituated to the enclosure (for about 10 minutes), and the behavior presented afterwards may rather reflect a general, spontaneous locomotor activity (38). With this in mind, there is a need to differently interpret increasing changes in SLA behavior, starting at 6 mg/kg ketamine and 10 mg/kg GL-II-73 with minor changes that hardly reflect any anxiety-inducing influence, up to ketamine at a dose of 10 mg/kg, at which a slight but consistent increase in emotional reactivity was revealed.

Our study was conducted in unstressed male rats with an intermittent administration regimen of ketamine. Wide variations in the effects of ketamine have been reported, related to dosage, treatment regimen, stress status, species, strain and sex of the animals, and time interval from the last dose (see 39,40). The stress-related dichotomy in the effects of ketamine appears to be particularly striking. It has been suggested that ketamine more reliably produces unwanted effects in non-stressed animals, whereas it has a beneficial effect in stressed animals (41). Fitzgerald *et al*. have shown that ketamine administered i.p. to C57BL/6J mice at two different doses (10 mg/kg and 30 mg/kg) produced depression-like behavior (decreased swimming and increased immobility) in the forced swim test in non-stressed rats 24 hours after injection, while the higher dose had the opposite effect in stressed rats, correcting the behavioral impairment caused by chronic, unpredictable stress (42). In another experiment, the same group showed no effect of 30 mg/kg i.p. ketamine in the forced swim test 72 hours after injection (43).

Similar phenomenon has been observed in humans. Namely, ketamine tends to exert prodepressive action in healthy volunteers, and the opposite in patients with major depressive disorder. This dichotomous effect has been documented in a series of randomized double-blind placebo-controlled studies led by the group of Carlos A. Zarate Jr. (44-47). They have shown that ketamine in healthy control subjects induced symptoms of anxiety, emotional blunting and anhedonia. This effect was related to increased gamma oscillations in multiple nodes related to the depression network, where presumably ketamine affected homeostatic plasticity in these regions, which resulted in altered homeostatic balance, and the effects were conditioned on the baseline functional state in the network (44). The authors also concluded that it differentially affected connectivity within the fronto-striatal network, which includes regions of the prefrontal cortex and striatum and is involved in cognition and affective processing (45). This contrasts with a previous report that postulated a global shift in connectivity (48). They also determined that the same effect of ketamine applies to functional connectivity during emotional processing tasks in healthy controls vs depressed patients (46,47).

It is important to note that there are studies on rats and mice that show behavioral changes in both directions and no change after ketamine injection in unstressed animals of the same strain (39,40). This variability is a recognized issue in the literature, and there are attempts to reach an explanation which connect it to different vendors for animal procurement, handling of experimental animals or even the sex of the experimenter (49).

Ketamine has been shown to induce anxiety in rats (50,51). Certain behaviours are sensitive to cumulative ketamine dose, suggesting a possible inverted U-shaped relationship (52). This aspect has not yet been adequately explored in ketamine research. Interestingly, there is an inverted U-shaped relationship between glutamate concentration and genetic excitation/inhibition (E/I) levels on the one hand and the temporal binding window (determinant of schizotypy) on the other, where both low and high E/I levels were associated with schizotypy and only optimal E/I levels contributed to favourable functioning (53). Intriguingly, this variability was shown in a cohort of healthy individuals, highlighting the importance of within-group variation that is overlooked in classical approaches.

Salome *et al*. compared the differences in cFOS expression after open field (OF) and open arm (OA) exposure in two lines of Wistar rats that were selectively bred for either high (HAB) or low (LAB) anxiety related behavior (54). They concluded that HAB rats had significantly higher cFOS expression in the medial and lateral hypothalamus and lower expression in the medial prefrontal cortex (mPFC) in response to both OF and OA exposure. These results indicate that the anxious behavior after ketamine injection in our experiment might be related to an mPFC mechanism.

Compelling line of evidence suggests that the influence of ketamine on interneurons (predominantly somatostatin (SST), or parvalbumin (PV) positive cells) is key for its function (55). α5GABAAR potentiation in our behavioral experiments mirrors, to a small extent, the proanxiety effects of ketamine. α5GABAARs are expressed in the prefrontal cortex on dendrites of pyramidal neurons innervated by SST+ interneurons and are key effectors of dendritic inhibition (56), and modulation of dendritic inhibition is an important target of ketamine (57). Very recent evidence has emerged that although ketamine initially decreases GABAergic function, contrary to traditional views it later increases GABAergic function, which is key for its sustained behavioral effects (27). It has also been shown that ketamine potentiates the function of extrasynaptic GABAARs (58,59). A single 10 mg/kg GL-II-73 dose can induce anxiolytic response in the elevated plus maze in unstressed mice (21). Also, a previous report from our group demonstrated a dose dependent bimodal influence of a different α5GABAAR PAM on various forms of memory, suggesting that α5GABAAR potentiation, similar to ketamine displays a very sensitive range for producing optimal effects (60). Additional studies are necessary to parse out the precise influence of α5GABAAR potentiation on anxiety. Opto-

genetic activation of excitatory neurons in the orbitofrontal cortex (OFC) induced increased anxiety, while activation of inhibitory neurons had an opposite effect (61). Also, fiber photometry of OFC neurons demonstrated lower activity of excitatory neurons during exposure to various anxiogenic stimuli including the center of the OF arena (61).

CONCLUSION

It is assumed that the mechanisms of ketamine and GABAAR modulators overlap to a certain extent. Intermittent administration of ketamine and prolonged α5GABAAR potentiation produced similar behavioral effects in rats. These results add to the growing literature suggesting that agents targeting the glutamate/GABA system may not appear to have beneficial effects in non-stressed rats. Finally, these results support the postulate that emotional reactivity has a sensitive relationship with the E/I state.

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Conflict of interest: J.M.C. and M.M.S. are listed inventors on patents covering the synthesis and use of the compound.

Author contributions: The conception or design of the work, M.M.S.; The acquisition, analysis, or interpretation of data: all authors; Preparing the draft of the manuscript or interpretation of revised version of manuscript, Dj.Dj., J.I., K.J., J.A., M.M.S.; All authors have read and agreed to the submitted version of the manuscript.

Ethical approval: The research was conducted in accordance with European Union guidelines (Directive 2010/63/EU). It was approved by the Ethics Committee for Animal Experiments of the University of Belgrade – Faculty of Pharmacy, Serbia and the Ministry of Agriculture, Forestry and Water Management – Veterinary Directorate (323-07-10046/2020-05, 23/09/2020).

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DUGOTRAJNA BLOKADA NMDA RECEPTORA I POZITIVNA MODULACIJA α5 $GABA_A$ RECEPTORA KOD NESTRESIRANIH PACOVA: ODSUSTVO UTICAJA NA DEPRESIVNO PONAŠANJE UZ BLAGO POVEĆANJE EMOCIONALNE REAKTIVNOSTI POD UTICAJEM PRETHODNE

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Sažetak

Uvod: Depresija predstavlja tešku psihijatrijsku bolest sa ograničenim terapijskim repertoarom. Značajan prodor u istraživanju depresije predstavlja otkriće brzodelujućih antidepresiva, poput ketamina i neurosteroida. Pozitivna modulacija $GABA_A$ receptora koji sadrže α5 subjedinicu (α5GABAAR) predstavlja obećavajući novi pristup u ciljanoj terapiji depresije. Cilj ove studije bio je da se ispita dejstvo ponavljanog davanja ketamina, antagoniste NMDA receptora i GL-II-73, pozitivnog alosternog modulatora α5GABAAR na depresivno i anksiozno ponašanje, kao i lokomotornu aktivnost.

Materijal i metode: U eksperimentima su korišćeni adultni mužjaci *Sprague-Dawley* pacova. Životinje su izlagane sedmodnevnom tretmanu ketaminom svakog drugog dana u dozi od 6 mg/kg ili 10 mg/kg, dnevnoj primeni 10 mg/kg GL-II-73 ili rastvarača. Nakon tretmana sprovedena je baterija bihejvioralnih testova koja se sastojala od testa forsiranog plivanja (engl. forced swimm test-FST), spontane lokomotorne aktivnosti (engl. spontaneous locomotor activity - SLA) i testa supresije hranjenja (engl. novelty suppressed feeding test - NSFT).

Rezutati: Nijedan vid tretmana nije ostvario uticaj na ponašanje u FST i NSFT. U SLA, svi vidovi tretmana uzrokovali su smanjenje procenta vremena u centralnom delu prostora tokom 10 i 30 min praćenja.. Pređena distanca u centralnom delu prostora bila je smanjena samo kod 10 mg/kg ketamina tokom 10 i 30 min.

Zaključak: Ketamin u većoj dozi (10 mg/kg) prouzrokovao je promene ponašanja koje odgovaraju blago povećanoj emocionalnoj reaktivnosti, dok promene istog kvaliteta uočene nakon primene manje doze ketamina i u GL-II-73 grupi ne oličavaju jasan proanksiozni uticaj.

Ključne reči: depresija, anksioznost, ketamin, α5 $GABA_A$ receptorska modulacija

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ORIGINAL ARTICLE

Surgical treatment of carotid body tumors: a single-center retrospective study

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Summary

Introduction: This study presented our experience in the surgical treatment of carotid body tumors (CBT).

Material and Methods: We retrospectively analyzed patients who underwent carotid surgery between 2009 and 2021. CBT patients were identified, and a control group of patients without CBT was selected using systematic sampling. Demographic and clinical characteristics were compared. Statistical analysis included Student's t-test, Pearson's chi-squared test, and Fisher's exact test.

Results: Among 6728 patients operated for carotid pathology, 16 (0.24%) had CBT. Four CBT patients presented with compressive symptoms, while 12 had medium-sized tumors classified as Shamblin I or II. Subadventitial excision was performed in 12 cases; in four, carotid artery resection and reconstruction were necessary. CBT patients were significantly younger than controls (55.8 ± 17.9 vs. 69.2 ± 7.5 years, p = 0.009). The CBT group included fewer males (25% vs. 68.2%, p = 0.001) and fewer smokers (18.7% vs. 50%, p = 0,014). ASA physical status scores were significantly lower in the CBT group (p = 0.001), and hypertension was less prevalent (75% vs. 92.9%, p = 0.03).

Conclusion: Subadventitial resection of CBT with deliberate resection of the external carotid artery is a simple and efficient procedure. A resection of the internal carotid artery during CBT surgery requires immediate repair or replacement. Our findings indicate significant differences in demographic and clinical characteristics between patients with CBT and those undergoing carotid surgery for atherosclerotic disease, emphasizing the importance of careful assessment and individualized management of this specific patient group.

Keywords: Cervical paraganglioma, Carotid Body Tumor, Surgical Treatment.

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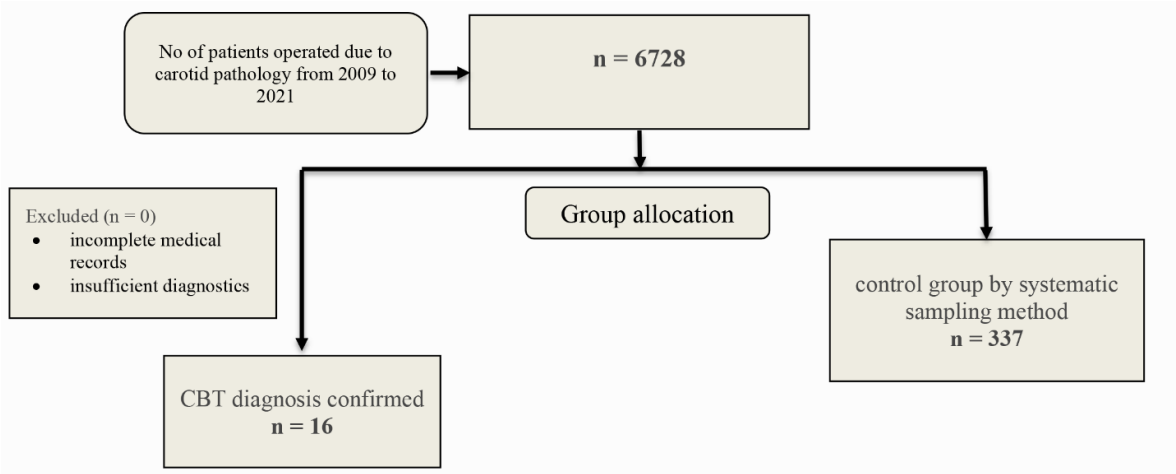

Figure 1. Flow Diagram of the Present Study.

INTRODUCTION

The carotid body is a type of chemoreceptor located at the medial portion of the carotid bifurcation, detecting changes in the level of oxygen, carbon dioxide, and pH in the blood (1). Carotid body tumors (CBT) are the most common paragangliomas of the neck region. Jugular bulb and tympanic paragangliomas are much rarer (2-4). These tumors are mostly benign, and surgical excision is the treatment of choice in the majority of cases. The main aim of this retrospective study was to analyze our experience of CBT's surgical treatment, i.e., to analyze the surgical outcomes of CBTs according to the Shamblin classification and to compare clinical and demographic characteristics of CBT patients against patients with other carotid pathologies.

MATERIAL AND METHODS

Consecutive patients who underwent surgery due to carotid pathology at the Clinic for Vascular and Endovascular Surgery of the University Clinic Center of Serbia between 2009 and 2021. were included in the study. Among them, patients with a pathohistologically confirmed diagnosis of CBT were identified. Conversely, the exclusion criteria implemented to maintain the integrity and robustness of the study's findings were: incomplete medical records or insufficient diagnostics **(Figure 1)**. Basic demographic and clinical data, along with clinically relevant characteristics of the disease, as well as data regarding the postoperative course, were obtained from patients' medical records. Additional postoperative data were gathered at regular postoperative check-ups. The decision for surgical treatment was established according to findings of color duplex scan (CDS) and multidetector computed tomography (MDCT) angiography examination **(Figure 2)**. All of those patients were operated on under general endotracheal anesthesia through longitudinal cervicotomy. Shamblin classification of tumors was established based on intraoperative findings **(Figure 3)**. Diagnosis of CBT was confirmed by pathohistological analysis of tumor samples, and the Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) was calculated to predict the

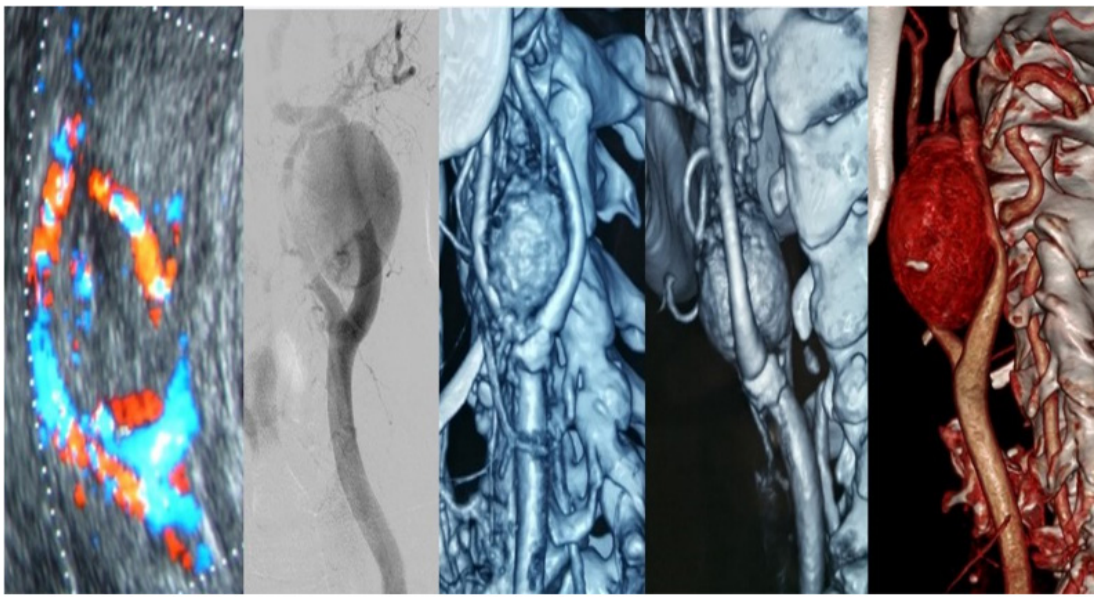

Figure 2. Diagnostic tools for carotid body tumor.

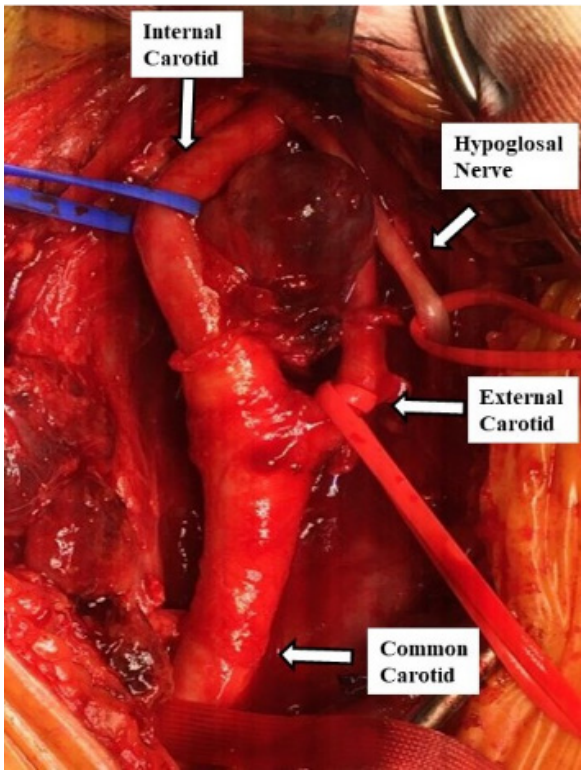

Figure 3. Intraoperative findings of a carotid body tumor.

potential malignant behavior of the tumor. According to the protocol of our Institution, patients were followed up by ultrasound (at one and six months following surgery, and annually thereafter) for five years. A follow-up of five years was completed in 9 patients, and the remaining were followed from 6 months to 2 years. Being that this is a retrospective study involving anonymized patient data, the requirement for ethical approval was waived by the institutional policy of the Clinic for Vascular and Endovascular Surgery, University Clinical Center of Serbia, and the principles outlined in the Declaration of Helsinki.

To compare demographic and clinical characteristics, a control group of patients without CBT who underwent carotid surgery was selected using systematic sampling. Starting from a randomly selected patient among the first 20, every 20th patient was included based on the chronological order of surgery, provided that complete data of interest were available. Relevant clinical and demographic variables were extracted from medical records and compared between the two groups.

Statistical analysis

Descriptive statistics methods were employed to analyze and interpret the collected data. Continuous variables are reported as means ± standard deviation (SD). Categorical variables are presented as absolute counts (n) and percentages (%). Continuous variables were compared between the groups using Student's t-test. In contrast, Pearson's chi-squared test or Fisher's exact test was used to compare the differences in the frequency of categorical variables. The level of significance was set at <0.05, and statistical analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA).

Being that this is a retrospective study involving anonymized patient data, the requirement for ethical approval was waived by the institutional policy of the Clinic for Vascular and Endovascular Surgery, University Clinical Center of Serbia, and the principles outlined in the Declaration of Helsinki. Shortly, this study has been conducted in full accordance with national and international ethical guidelines and standards relevant for this type of study. All statistical analyses were performed on anonymized patient data, ensuring that individual identities could not be disclosed or compromised

RESULTS

A total of 6728 patients were operated on due to carotid pathology from 2009 to 2021. Among them, 16 patients (0.24%) with CBT who underwent surgical treatment during these 12 years were identified. Basic characteristics of CBT patients are presented in Table 1, while Table 2 summarizes Shamblin classification, surgery characteristics, and PASS score. Male/female ratio was 1:3 (N male=4, N female=12). The mean age of CBT patients was 55.8 ± 17.9 years (range 23–82 years). In ten patients (63.5%), the tumor was right-sided, and no patients presented with bilateral tumors. In twelve patients (75%), the tumor presented as an asymptomatic neck mass.

In comparison, in four patients, the tumor caused symptoms of compression on surrounding structures (2 patients with dysphagia, one with odynophagia, and one with stridor). In twelve patients, the tumor was medium-sized and classified as Shamblin I and II. Others infiltrated the carotid arteries and were classified as Shamblin III. Subadventitial tumor excision was performed in 12 patients with Shamblin I and II, while in four patients with Shamblin III, carotid arteries were resected and then reconstructed. In three of them, reconstruction was performed with a prosthetic Dacron graft, and in one with significant internal carotid artery elongation, end-to-end anastomosis was performed. In five patients (31%), enlarged local lymph nodes were found. They were subjected to pathohistological analysis, which showed hyperplastic enlargement. None of our patients had systemic metastasis according to the findings of preoperative MDCT.

After surgical treatment, transient damage to the hypoglossal nerve was found in two patients. There were no postoperative complications such as hematoma, stroke, or Horner syndrome. Only one patient had a pathological PASS score higher than 3, which was established as borderline for malignancy. That patient had a PASS score of 8. Also, there was no in-hospital mortality. Postoperative follow-up and control color Doppler sonography were performed in all patients. Control angiography was performed in 7 patients (43.8%). We did not find any tumor recurrence.

Table 1. Basic characteristics of patients with carotid body tumor, symptoms, and diagnosis

Patients' characteristics	Number (Percentage)
Age - mean (years)	55.8 ± 17.9
Sex - male	4 (25%)
Preoperative symptomatology - yes	4 (25%)
Tumor side - left	10 (63.5%)
Diagnosis	
Angiography	7 (43.7%)
Duplex + angiography	9 (56.3%)

Table 2. Shamblin classification, surgery characteristics, and Pheochromocytoma of the Adrenal Gland Scaled Score

	Number (Percentage)
Shamblin classification	
I	3 (18.7%)
II	9 (56.3%)
III	4 (25%)
Tumor-related procedure - excision	16 (100%)
Vascular procedure	
No	12 (75%)
Resection + synthetic graft	3 (18.7%)
Resection + end-to-end anastomosis	1 (6.3%)
Complications	
No	14 (87.5%)
Hoarseness	2 (12.5%)
PASS score	
III	15 (93.7%)
VIII	1 (6.3%)

PASS score -Pheochromocytoma of the Adrenal Gland Scaled Score

Using a systematic sampling method, a control group of 337 patients who underwent carotid surgery without CBT was identified during these 12 years. Patients in the CBT group were significantly younger compared to the control group (the mean age 55.8 ± 17.9 years vs. 69.2 ± 7.5, p = 0.009). Furthermore, the difference in sex distribution was also statistically significant: there were four male patients in the CBT group (25%), compared to 230 males (68.2%) in the control group (p = 0.001). When the two groups were compared according to body mass index, no significant differences were noted: 26.3 ± 3.7 kg/m^2 (control group) vs. 27.5 ± 4.2 kg/m^2 (CBT group), p = 0.42. Similarly, no significant differences were observed when the groups were compared in terms of symptomatology. In the CBT group, four patients (25%) presented with symptoms, while 131 (38.8%) were symptomatic in the control group (p = 0.261). Significantly fewer patients were active smokers in CBT than in the control group (18.7% vs. 50%, p = 0.014). The American Society of Anesthesiologists (ASA) physical status score was also significantly higher in the control group compared to CBT patients: ASA 3 score was noted in 90.5% in the control group vs. 12.5% in the CBT group (p = 0.001). Significantly more patients in the control group had hypertension, compared to the CBT group (92.9% vs. 75%, p = 0.03). Still, no significant differences were noted when those two groups were compared in terms of diabetes mellitus (36.5% vs. 31.3%, p = 0.794).

DISCUSSION

Carotid body tumors (CBT) are extremely rare and highly vascularized (5). The arterial supply typically derives from the ascending pharyngeal and occipital branches of the external carotid artery. However, contributions from the internal carotid artery, vertebral artery, and thyrocervical trunk can develop (6). Due to this, surgical treatment of the CBT is associated with significant bleeding, which at the same time, makes it more difficult or even disables tumor removal, increasing the risk of iatrogenic injuries to surrounding structures (7). According to the same reports, preoperative embolization reduces intraoperative blood loss and decreases technical difficulties during surgical removal of carotid body tumors (8-12). To be more precise, a selective preoperative embolization includes the major feeding arteries that originate from the external carotid artery. It requires a very precise technique to prevent potential central neurologic complications (13). The role of preoperative embolization before surgical treatment of CBT is controversial (14-17). We do not use it before CBT surgery.

The incidence of bilateral CBT is 5% (18-20). The tumor is three times frequent in female patients, and patients are mostly in their 5th decade (21). Five to 9% of patients with CBT have a positive family history. An equal distribution between men and women in patients with familial CBT supports an autosomal mode of genetic transmission (22). We did not have patients with bilateral and familial CBT, while the mean age of treated patients was 55.8 years.

The majority of CBT grows slowly and has benign characteristics (23-30). The malignant potential of these tumors is controversial (31, 32). The reported rates of malignancy range from 2% to 50% (23-30). The metastatic rate of CBT is approximately 5% (23-30). The metastatic spread generally occurs in regional lymph nodes. However, systemic metastases that involve the kidney, thyroid, pancreas, cerebellum, lungs, bone, brachial plexus, abdomen, and breast are also described (23-30). In our study, there were no CBTs with malignant characteristics.

An asymptomatic, rubbery, firm, and non-compressible neck mass located below the angle of the mandible is the most common clinical presentation of the CBT (33-37). The mass is often pulsatile due to its close relationship with the carotid artery. Other nonspecific symptoms may include neck or ear pain, as well as compression abnormalities caused by vagal, hypoglossal, cervical sympathetic, and esophageal involvement (20, 33-37). Approximately 5% of CBT may have neurosecretory activity. Due to this, some patients describe symptoms of hypertension, tachycardia, arrhythmias, headache, and so on (20, 37-

40). CBT's intracranial extension can cause lateralizing and central neurologic symptoms (38). Death due to asphyxia in patients with CTB is described as well (39, 40). The majority of our patients were asymptomatic, but four patients presented with symptoms of surrounding tissue compression. For instance, CBT patients may experience vocal cord paralysis, facial palsy, and ischemic stroke (41).

In all patients with neck mass located below the angle of the mandible, the initial diagnosis should begin with duplex ultrasonography. If CBT is suspected, the diagnosis continues with MDCT angiography (10, 19, 20, 37, 42). Characteristically, a carotid body tumor separates the internal and external carotid arteries, widening the bifurcation (10, 19, 20, 37, 42). In addition, diagnostic procedures should provide information about the concomitant carotid occlusive disease. Since MDCT angiography has been introduced, standard angiography has lost its importance for the diagnosis of CBT. In all our patients, the final diagnosis has been established using MDCT angiography.

Some authors have reported palliation using radiation therapy alone in patients with CBT. However, radiation therapy can be followed by significant local complications, including osteonecrosis of the mandible, carotid radiation arteritis, and laryngeal nerve injury (25, 30, 33).

Most agree that surgery is the method of choice for the treatment of CBT (43). Reigner performed the first resection of the CBT in 1880, but his patient did not survive (44). Six years later, Maydl removed a carotid body tumor, but the patient became aphasic and hemiplegic (45). Finally, in 1903, Scudder performed the first successful removal of a CBT with preservation of the carotid arteries as well as surrounding cranial and cervical nerves (46).

General anesthesia is preferable during CBT surgery, and we applied this anesthesia technique in all our patients. Patient positioning is identical to that for carotid endarterectomy (18, 47, 48). A procedure begins with a longitudinal skin incision along the anterior border of the sternocleidomastoid muscle (47, 48). In the case of large CBT, a modified-T neck incision is recommended (18). Surgical treatment of CBT can be complicated because of its intimate relationship with the carotid arteries and cranial and cervical nerves. However, not all tumors are equally challenging for surgical treatment.

Regarding that, Shamblin classified CBTs into three groups (18, 25). *Group I* includes relatively small tumors, which are minimally attached to the carotid vessels. Due to this, surgical excision is not difficult (18, 25, 47, 48). *Group II* includes larger tumors with moderate attachments to the carotid arteries. Finally, *group III* includes massive tumors that encase the carotid arteries (18, 25, 47, 48). The first step in surgical treatment of CBT involves proximal and distal bleeding control, which requires isolating the standard, external, and internal carotid arteries with surgical tapes (18, 19, 37). It is recommended to isolate the external and internal carotid arteries above the upper pole of the tumor. The next step is identifying the hypoglossal and vagal nerves that must be protected during tumor dissection.

In cases with tumors Shamblin types I and II, a dissection proceeds in a subadventitial plane along the carotid arteries (10, 18, 37, 42, 49). Gordon Taylor introduced subadventitial dissection during the removal of the CBT in 1940 (50). The injury of cranial and cervical nerves may be avoided by meticulous dissection around the tumor and by the use of bipolar electrocautery to minimize the conductive heat effect. Temporary clamping of the external carotid artery in patients with Shamblin II reduces bleeding during tumor dissection (10, 18, 37, 42). The Shamblin III of CBT requires resection of the external and/or internal carotid artery (18, 19, 37, 48, 51). In such cases, earlier ligation and division of the external carotid artery reduce bleeding and facilitate further resection of the tumor. Reconstruction of the external carotid artery following tumor removal is not necessary. However, ligation of the internal carotid artery results in a stroke incidence ranging from 23% to 50% and a mortality rate of 14% to 64% (52). Due to this, an excision of the internal carotid artery that is involved with the tumor requires its immediate repair/replacement with end-to-end anastomosis or vascular graft (18, 19, 37, 48, 51). Three of our patients required graft replacement of the carotid artery after tumor excision. Still, in one patient with significant internal carotid artery elongation, reconstruction was done with end-to-end anastomosis. If prolonged clamping and reconstruction of the internal carotid artery is necessary during CBT surgery, the same authors recommend the use of the carotid shunt (19). However, ipsilateral hemispheric hypoperfusion after internal carotid artery clamping during CBT surgery is an infrequent cause of neurological events. Due to this, we do not use carotid shunts during CBT surgery.

Advancements in subadventitial dissection and vascular surgery techniques have significantly decreased the perioperative stroke occurrence after CBT surgery from 30% to below 1%. At the same time, perioperative mortality, which was 50% five decades ago, has decreased to 0% in nowadays studies (2, 18, 19, 30, 37, 42, 51). Unlike central neurologic complications, the incidence of local neurologic complications (iatrogenic injuries of surrounding cranial and cervical nerves) after CBT surgery remained relatively significant (53, 54). In the most recently published papers, the incidence of these injuries is nearly 10% (2, 5, 19, 55-57). Transient hypoglossal nerve injury occurred in one of our patients (16.67%). Our previous reports included 17 cases of CBT treated between 1982 and 2007 (47, 48). Perioperative stroke rate and cranial nerve injuries were 6% without perioperative mortality.

Finally, the present study highlights several important clinical characteristics of patients with CBT that warrant attention. Our results show that CBT patients exhibit distinct profiles compared to individuals undergoing carotid

artery surgery for atherosclerotic disease. Notably, CBT patients are significantly younger and more frequently female. They also have a lower prevalence of smoking and hypertension, and generally present with a better ASA physical status score. These differences likely reflect the non-atherosclerotic, often benign nature of CBT, which is typically of neuroendocrine origin and may be influenced by genetic or familial factors rather than conventional cardiovascular risk factors (58).

In contrast, patients undergoing carotid revascularization for atherosclerotic disease tend to be older and have a higher burden of comorbidities, such as smoking, hypertension, and cardiovascular disease (59). Interestingly, no significant differences were observed between the groups in terms of BMI, presence of symptoms, or the prevalence of diabetes mellitus. This suggests that, while some clinical and metabolic features may overlap, CBT patients constitute a fundamentally different population. Recognizing these distinctions is essential not only for accurate surgical risk stratification but also for optimizing preoperative evaluation and tailoring long-term follow-up strategies.

This study has several limitations that should be taken into consideration. Most notably, the retrospective design of the present study limits the level of control over data collection, as the analysis relies solely on pre-existing medical records. The relatively small number of patients (due to the low incidence of CBT) restricts the generalizability to broader populations. Therefore, the results should be interpreted with caution. Nevertheless, the study provides critical observational data on the surgical management of rare carotid body tumors and has observational and hypothesis-generating potential for future studies.

CONCLUSIONS

CBTs are rare and mostly benign. Surgery is the method of choice for the treatment of these tumors. Subadventitial resection of CBT with deliberate resection of the external carotid artery is an efficient procedure for the surgical management of this tumor. A resection of the internal carotid artery during CBT surgery requires immediate repair or replacement. Our findings indicate a marked difference in demographic and clinical characteristics between patients with CBT and those undergoing carotid surgery for atherosclerotic disease, highlighting the need for careful evaluation and individualized management of this unique patient population.

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Ethical approval: Being that this is a retrospective study involving anonymized patient data, the requirement for ethical approval was waived by the institutional policy of the Clinic for Vascular and Endovascular Surgery, University Clinical Center of Serbia, and the principles outlined in the Declaration of Helsinki. Shortly, this study has been conducted in full accordance with national and international ethical guidelines and standards relevant for this type of study. All statistical analyses were performed on anonymized patient data, ensuring that individual identities could not be disclosed or compromised.

Informed consent: Informed consent was obtained from all subjects with carotid body tumors involved in the study.

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HIRURŠKO LEČENJE TUMORA KAROTIDNOG TELAŠCA: RETROSPEKTIVNA STUDIJA U OKVIRU JEDNOG CENTRA

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Sažetak

Uvod: Ova retrospektivna studija prikazuje iskustva tercijarnog centra u hirurškom lečenju bolesnika sa tumorom karotidnog telašca (glomus tumorom).

Metode: Analizirani su pacijenti koji su podvrgnuti karotidnoj hirurgiji u periodu 2009–2021. Identifikovani su pacijenti sa glomus tumorom, a kontrolna grupa bez tumora selektovana je sistematskim uzorkovanjem. Upoređene su demografske i kliničke karakteristike među grupama. Za statističku analizu korišćeni su Studentov t-test, Pirsonov hi-kvadrat test i Fišerov test tačne verovatnoće.

Rezultati: Među 6728 operisanih, kod 16 (0,24%) dijagnostikovan je glomus tumor. Kod četiri bolesnika, tumor je izazivao kompresivne simptome, dok je kod 12 klasifikovan kao *Shamblin* I/II. Kod 12 bolesnika urađena je subadventicijska ekscizija tumora, dok je kod četiri bila neophodna resekcija i rekonstrukcija karotidnih arterija. Pacijenti sa glomus tumorom bili su značajno mlađi od kontrolne grupe (55,8 ± 17,9 godina naspram 69,2 ± 7,5 godina, p = 0,009), sa manje muškaraca (25% naspram 68,2%, p = 0,001) i pušača (18,7% naspram 50%, p = 0,014). ASA fizikalni status bio je značajno bolji kod pacijenata sa glomus tumorom (p = 0,001), a hipertenzija je bila ređa (75% naspram 92,9%, p = 0,03).

Zaključak: Subadventicijalna resekcija glomus tumora sa resekcijom spoljašnje karotidne arterije predstavlja efektivni vid hirurškog lečenja ovih bolesnika. Resekcija unutrašnje karotidne arterije tokom ovih operacija zahteva istovremenu rekonstrukciju. Naši rezultati ukazuju na značajne demografske i kliničke razlike između pacijenata sa glomus tumorom i onih koji se podvrgavaju karotidnoj hirurgiji zbog aterosklerotske bolesti, naglašavajući značaj pažljive procene i individualizovanog pristupa u tretmanu ove specifične grupe pacijenata.

Ključne reči: Paragangliom, tumor karotidnog telašca; glomus tumor; hirurško lečenje

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ORIGINAL ARTICLE

Impact of physical activity on the progression of atherosclerosis in patients with antiphospholipid syndrome

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Summary

Introduction: Antiphospholipid syndrome (APS) is linked to an increased risk of cardiovascular diseases due to the interplay between traditional risk factors and antibody-induced endothelial damage. Limited data exist on the level of physical activity (PA) in APS patients. This study aimed to analyze PA domains in Serbian APS patients and their association with atherosclerosis progression.

Materials and Methods: Fifty-one APS patients (29 with primary APS (PAPS), mean age 44.0 ± 11.5, and 22 with APS/SLE (sAPS), mean age 48.4 ± 11.7) completed the long form of the International Physical Activity Questionnaire (IPAQ), translated into Serbian. PA was categorized into leisure time, domestic activities, work-related, and transport-related domains, and classified as low, moderate, or high. Carotid Doppler ultrasound scans were performed to detect atherosclerotic plaques.

Results: The total PA in a PAPS group ranged from 0 to 26.880 MET-minutes/week and in sAPS from 0 to 19.200. Work-related and domestic activities had the highest scores, while leisure time PA was lowest. Most patients had low (37.3%) or moderate (43.1%) PA levels, with only 19.6% in the high PA category. No significant PA differences were found between PAPS and sAPS patients. However, PAPS patients were more likely to have high PA (27.6% vs. 9.1%), though not significantly. Patients with lower PA scores had a higher prevalence of carotid plaques, particularly for vigorous (p=0.001) and total PA (p=0.002).

Conclusion: Younger Serbian APS patients predominantly exhibit low or moderate PA, especially during leisure time. Low PA strongly correlates with atherosclerosis progression, underscoring the need to promote physical activity in this population.

Keywords: antiphospholipid syndrome; atherosclerosis; physical activity.

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INTRODUCTION

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized clinically by recurrent thrombosis (venous and/or arterial) and/or recurrent spontaneous abortions, in conjunction with persistently elevated antiphospholipid antibodies (defined as a significant increase in these antibodies at least once every 12 weeks) (1). This syndrome can manifest either as a primary condition (PAPS) or be associated with other diseases (sAPS), including autoimmune, hematological, infectious, malignant, or neurological disorders. Antiphospholipid antibodies (aPL) constitute a family of autoantibodies with an affinity for negatively charged phospholipids complexed with phospholipid-binding proteins, and they are closely linked to the development of arterial and/or venous thrombosis, as well as obstetric complications.

The prevalence of APS is higher among women than men, with a ratio of 5:1, and it exhibits no predisposition concerning ethnicity. The annual incidence stands at 5 cases per 100,000 individuals, while the overall prevalence ranges between 40 and 50 cases per 100,000 people (2).

Circulating aPL often exhibit limited responsiveness to immunomodulatory treatments (3). Their persistent presence, often in varying titers, triggers numerous pathophysiological mechanisms (4). The chronic vasculopathy associated with APS is primarily characterized by endothelial dysfunction as a central hallmark of vascular abnormalities (5). Subsequent stages of vascular involvement lead to the progressive thickening of the intima, possibly due to endothelial cell proliferation (6-8). These vascular changes related to APS have initially been observed in the renal microvasculature (9,10). Similar occlusive lesions are likely under-recognized in organs and tissues that are not routinely subjected to biopsy, such as the brain, heart, and mesentery. Cerebrovascular and cardiovascular disorders, specifically stroke and myocardial infarction, are the leading causes of morbidity and mortality in APS patients, especially among young individuals. Primary APS patients have demonstrated that the risk of (sub)clinical atherosclerosis is associated with APS itself, independent of other underlying conditions (11).

Engaging in physical activity is associated with a lower risk of atherosclerotic cardiovascular disease, with greater volume and intensity of exercise leading to further reductions in risk. Research indicates that the most significant decline in risk occurs when transitioning from a sedentary lifestyle to approximately 5000 MET-minutes per week, equivalent to around 13–14 hours of walking weekly. This activity level is linked to an estimated 30% reduction in risk. Beyond this threshold, the benefits tend to plateau, with the maximum expected risk reduction reaching approximately 40–50% (12).

To support cardiovascular health, the World Health Organization (WHO) currently advises engaging in 150–300 minutes of moderate-intensity aerobic exercise or 75–150 minutes of vigorous-intensity exercise per week (13). In clinical settings, recommending an increase in daily step count may be a practical approach. Evidence suggests that adding 1000 steps per day—starting from a baseline of 2000 to 5000 steps—is associated with a 5–10% reduction in risk, with benefits continuing up to a total of 10,000 daily steps (14). Given the clear evidence of accelerated atherosclerosis in patients with APS, promoting physical activity in this population is of significant importance.

The primary objective of this study is to assess the levels of physical activity within a cohort of Serbian APS patients and investigate its potential correlation with the progression of the atherosclerotic process, as indicated by the presence of atherosclerotic plaques in the carotid arteries, as determined by Doppler ultrasound examination.

MATERIALS AND METHODS

This cross-sectional cohort study enrolled 51 APS patients between October 1, 2022, and January 31, 2023. The diagnosis of APS was established following the revised Sydney criteria (both laboratory and clinical) from 2006 (15). Diagnosis of Systemic Lupus Erythematosus (SLE) was made by a rheumatologist following the revised ARA (American College of Rheumatology) criteria (16). Disease activity was assessed at the time of enrollment using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (17). This study included 29 PAPS patients (25 females and 4 males, mean age 44.0±11.5) and 22 sAPS patients (18 females and 4 males, mean age 48.4±11.7). Exclusion criteria comprised conditions that could influence endothelial perturbation (acute or chronic infection, malignancy, or marked renal, cardiac, and hepatic impairment), as well as a diagnosis made less than 6 months prior. SLE patients lacked active central nervous system lupus manifestations, lupus nephritis, cytopenias, or any skin manifestations. The mean SLEDAI for SAPS patients was 4.7 ± 1.9. All patients received appropriate therapy in accordance with the activity of the disease (18).

The study obtained approval from the University Hospital Clinical Centre Bezanijska kosa Ethical committee (7673/1/2021; date: 19.10.2021.), and written informed consent was obtained from all individual participants. All procedures performed on patients adhered to the Helsinki Declaration and its subsequent amendments or equivalent ethical standards.

Patients

During routine outpatient visits, all patients underwent the following procedures: physical examination and electrocardiography (ECG) recording, Doppler carotid ultrasound, blood sampling, and assessment of physical activity via questionnaire.

In the clinical examination, the presence of standard risk factors for atherosclerosis was determined. Hypertension was defined as systolic blood pressure greater than 140 mmHg and/or diastolic blood pressure greater than 90 mmHg recorded on two or more consecutive examinations before initiating antihypertensive therapy. Body weight and height were utilized to calculate the body mass index (BMI) using Adolphe Quetelet's formula (height/weight^2). A normal BMI value was considered below 26 kg/m^2, while a BMI of 26-30 kg/m^2 indicated overweight, and a BMI above 30 kg/m^2 categorized patients as obese. Diabetes mellitus was diagnosed by an endocrinologist (19). Hypercholesterolemia was defined as a total cholesterol value exceeding 5.2 mmol/L on two or more consecutive measurements or if the patient was already receiving lipid-lowering drug therapy. Smoking habits were also recorded.

Carotid artery ultrasound examinations, encompassing both common and internal carotid arteries, were performed using a high-resolution 7.0 MHz linear probe equipped with a 4 MHz pulsed color Doppler for flow measurement (Acuson, Sequoya 216). Carotid arteries were visualized from two lateral and one transverse section. Patients were positioned supine with head extension and rotated at a 45-degree angle to the opposite side of the examined artery. The left and right common carotid arteries (1 cm proximal to the bifurcation), the left and right carotid bifurcations, and the left and right internal carotid arteries (1–2 cm distal to the bifurcation) were examined for the presence of atherosclerotic plaques (20). The examinations were conducted by the same experienced operator who was unaware of the subjects' diagnoses.

All patients underwent laboratory blood tests to detect aPL, as well as routine biochemical blood tests and a complete blood count. Lupus anticoagulant (LA) was tested following the International Society on Thrombosis and Haemostasis guidelines, while anticardiolipin antibodies (aCL) and/or anti-beta2glycoprotein I (anti-β2GPI) antibodies of IgG and/or IgM isotype at medium or high titers were measured using standardized ELISA (21). LA testing was not performed on patients receiving anticoagulant therapy. Anticardiolipin (aCL: IgG/IgM) and anti-ß2glycoprotein I (ß2GPI: IgG/IgM) antibodies were determined by enzyme-linked immunosorbent assay (ELISA) and expressed in GPL or phospholipid (MPL) units (GPL-U and MPL-U). Following the revised laboratory criteria for APS diagnosis, the analysis of aPL was repeated at an interval of at least 12 weeks (12). All APS patients were categorized into the following categories: category I when more than one laboratory criterion was present in any combination, category IIa when only LA was present, category IIb when only anticardiolipin antibodies (aCL) were present, and category IIc when only anti-β2 glycoprotein-I antibodies (anti-β2GPI) were present (22).

The level of physical activity was assessed using The International Physical Activity Questionnaire (IPAQ) for evaluating physical activity levels over the past week, translated into Serbian (Appendix I). The physical activity level was scored as low, medium, or high, and MET-min/week was calculated according to the current protocol (https://sites.google.com/view/ipaq/score#h.sqdsh530qc5o).

Statistical Analysis

Descriptive and analytical statistical methods were employed in this study. Descriptive methods included absolute and relative numbers (n, %), measures of central tendency (arithmetic mean, median), and measures of dispersion (standard deviation). Analytical methods involved difference tests, both parametric (t-test) and non-parametric (Pearson's chi-square test, Fisher's exact probability test, Mann-Whitney U test). The choice of test depended on the data type and distribution. Parametric methods were used when the distribution was normal, while non-parametric methods were used for non-normal distributions. Distribution normality was assessed based on descriptive parameters, normality tests (Kolmogorov-Smirnov and Shapiro-Wilks test), and graphical methods (histogram, box plot, QQ plot). Univariate and multivariate logistic regression analysis was employed for association analysis. The level of statistical significance was set at $p<0.05$. All data were processed using SPSS 20.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Table 1 presents the clinical and laboratory characteristics of both patient groups. Patients with PAPS were significantly more likely to exhibit obstetric APS (69.0% vs. 36.4% in the sAPS group, p=0.022). Conversely, patients in the sAPS group were substantially more likely to possess positive aCL IgG (54.5% vs. 27.6% in the PAPS group, p=0.053), anti β2 GPI IgG antibodies (50.0% vs. 24.1% in the PAPS group, p=0.058) and triple aPL positivity (40.9% vs 13.8%, p=0.029). However, there were no differences in age, gender, and the prevalence of standard atherosclerotic risk factors between these two groups of subjects. Notably, the prevalence of standard atherosclerotic risk factors was less than 40% for both groups of respondents.

Regarding the different types of PA including a total time spent, there was no statistically significant difference between PAPS and sAPS patients. These results are summarized in **Table 2**.

While a notably higher percentage of PAPS patients exhibited a high level of physical activity (27.6% vs. 9.1%), statistically significant differences in the various levels of physical activity (low, medium, and high) were not observed between the examined groups (p=0.129). **Figure 1** provides a graphical representation of these findings.

Table 1. Demographic and clinical features of PAPS and sAPS patients

Variable	PAPS (n=29)	sAPS (n=22)	p-value
Age	44.0± 11.5	48.4 ± 11.7	0.185
Gender (m/f)	(4/25)	(4/18)	0.713
BMI (kg/m2)	25.0 (18.3-27.8)	25.1 (22.5-28.1)	0.549
Standard atherosclerotic risk factors			
Arterial Hypertension	5 (17.2%)	9 (40.9%)	0.063
Diabetes mellitus	1 (3.4%)	0 (0.0%)	0.384
Smoking	9 (31.0%)	6 (27.3%)	0.772
Hyperlipidemia	6 (20.7%)	8 (36.4%)	0.219
Specific clinical features			
Obstetric APS	20 (69.0%)	8 (36.4%)	**0.022**
Thrombosis	11 (37.9%)	12 (54.5%)	0.242
Arterial thrombosis	6 (20.7%)	10 (45.5%)	0.062
Venous thrombosis	5 (17.2%)	5 (22.7%)	0.628
apL characteristics			
LA	17 (58.6%)	13 (59.1%)	0.973
aCL IgG	8 (27.6%)	12 (54.5%)	0.053
aCL IgM	11 (37.9%)	11 (50.0%)	0.393
anti β2 GPI IgG	7 (24.1%)	11 (50.0%)	0.058
anti β2 GPI IgM	13 (44.8%)	12 (54.5%)	0.496
Category I	20 (69.0%)	17 (77.3%)	
Category IIa	5 (17.2%)	3 (13.6%)	0.489
Category IIb	3 (10.3%)	2 (9.1%)	
Category IIc	1 (3.4%)	0 (0.0%)	
Triple apL positivity	4 (13.8%)	9 (40.9%)	**0.029**

PAPS: primary antiphospholipid syndrome, sAPS: antiphospholipid syndrome associated with systemic lupus erythematosus, LA – lupus anticoagulant, apL – antiphospholipid antibody, aCL – anticardiolipin antibody GPI – glycoprotein1

Table 2. Level and type of physical activity in antiphospolipid syndrome patients

Variable	PAPS (n=29)	sAPS (n=22)	p-value
Total physical activity (MET-min/weekly)	0 (0-29.454)	0 (0-20.109)	0.177
Working place(MET-min/ weekly)	0 (0-26.880)	0 (0-19.447)	0.138
In-home activities (MET-min/ weekly)	1.080 (0-21.000)	900 (0-7920)	0.379
Leisure (MET-min/ weekly)	231 (0-2910)	297.5 (0-2772)	0.497
Transport (MET-min/ weekly)	693 (0.0-6930)	214.5 (0-2.772)	0.390
Walking (MET-min/ weekly)	1386 (0-6930)	528 (0-5544)	0.226
Moderate physical activity (MET-min/ weekly)	0 (0-14.400)	0 (0-5040)	0.210
Vigorous physical activity (MET-min/ weekly)	0 (0-26.880)	0 (0-19.200)	0.382

PAPS: primary antiphospholipid syndrome, sAPS: antiphospholipid syndrome associated with systemic lupus erythematosus

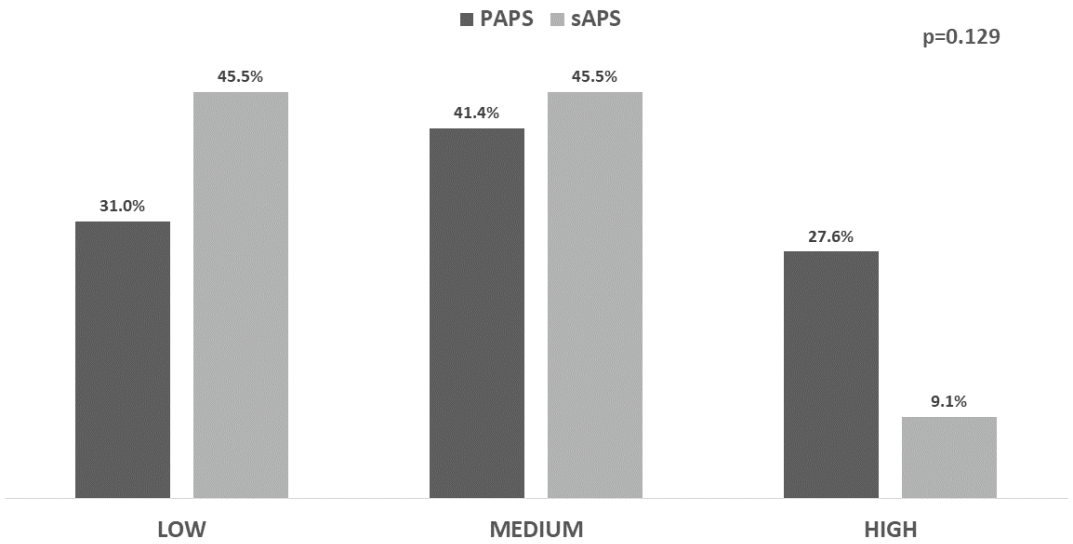

Figure 1. Level of physical activity among groups

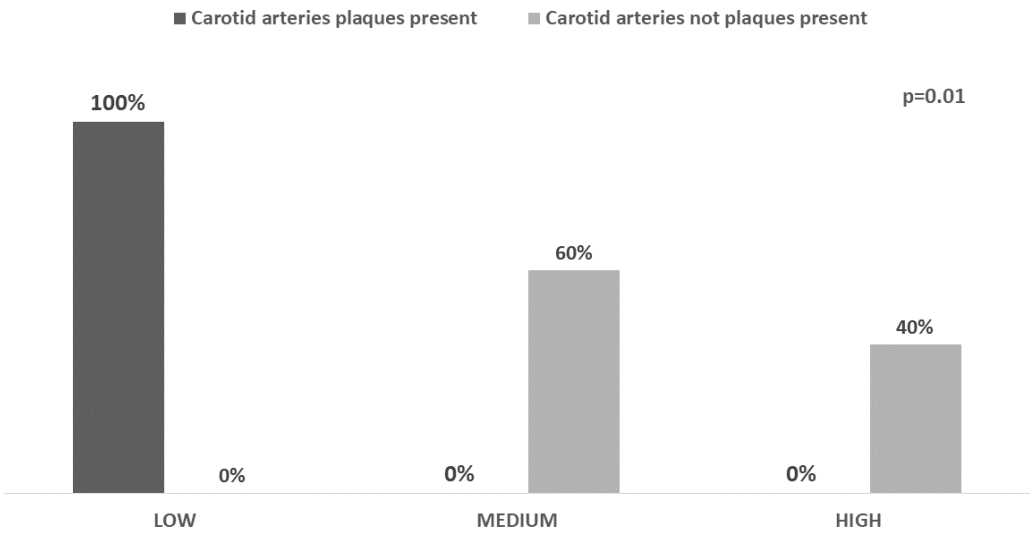

Figure 2. Level of physical activity considering carotid atherosclerotic plaque presence

The presence of carotid artery plaques was highly statistically significantly correlated with the level of PA in APS patients (p=0.01). All APS patients with plaques fell into the category of low physical activity. **Figure 2** visually represents these results.

APS patients with carotid artery plaques spent significantly fewer minutes engaged in physical activity at the workplace, during transportation, walking and in vigorous activities, as well as in total physical activity (p=0.042, p=0.011, p=0.021, p=0.001, p=0.002, respectively). **Table 3** provides a detailed presentation of these findings.

DISCUSSION

To date, there has been limited exploration of the impact of PA on APS patients in the literature. Our study sheds light on the fact that APS patients tend to have low levels of PA, and we have established a significant association between greater PA and a reduced prevalence of atherosclerotic plaques in the carotid arteries. This is particularly crucial given the predisposition of APS patients to cardiovascular diseases.

Karakis et al. demonstrated that APS patients had significantly elevated values of common carotid artery intima-media thickness (IMT), internal carotid artery IMT, and carotid bifurcation IMT, and they were more frequently diagnosed with atherosclerotic plaques compared to controls (11). Due to the heightened risk of atherosclerosis, the authors concluded that these patients require early and disease-specific atherosclerosis prevention. APS patients have been reported to have a 2.5–4-fold higher risk of subclinical atherosclerosis compared to the general population (11,23), a risk comparable to that seen in patients with diabetes mellitus (24, 25). Traditional risk factors such as hypertension and dyslipidemia, in combination with non-traditional factors including antiphospholipid antibodies (aPL), inflammation, and low anti-phosphorylcholine levels, contribute to the elevated risk of cardiovascular disease in Systemic Lupus Erythematosus (SLE) (26, 27).

Common risk factors for atherosclerosis typically encompass elevated cholesterol and triglyceride levels, high blood pressure, smoking, diabetes mellitus, obesity, and a sedentary lifestyle. Despite continuous advancements in therapeutic methods to manage these risk factors, the global prevalence of cardiovascular diseases remains high. Alarmingly, awareness of the risk of developing or exacerbating cardiac disorders in individuals with APS or other autoimmune diseases is often suboptimal. Bolla et al. conducted a study on 61 APS patients and found a similar or even higher prevalence of traditional cardiovascu-

Table 3. Time of the physical activity considering carotid atherosclerotic plaque presence

	Carotid artery plaque presence		
	Yes	No	p-value
Total physical activity (MET-min/weekly)	2580 (0-24.390)	8379 (0-29.454)	**0.002**
Working place (MET-min/ weekly)	0 (0-19.200)	693 (0-26.880)	**0.042**
In-home activities (MET-min/ weekly)	855 (0-11.040)	1890 (0/21.00)	0.328
Leisure (MET-min/ weekly)	99 (0-2097)	693 (0-2910)	0.072
Transport (MET-min/ weekly)	214.5 (0-6930)	792 (693-2772)	**0.011**
Walking (MET-min/ weekly)	561 (0-6930)	2079 (2079-6276)	**0.021**
Moderate physical activity (MET-min/ weekly)	0 (0-14.400)	0 (0-10.500)	0.851
Vigorous physical activity (MET-min/ weekly)	0 (0-19.200)	0 (0-26.880)	**0.001**

lar risk factors in APS compared to patients with rheumatoid disease and diabetes mellitus (28). Their study also hinted at an improvement in the control of certain risk factors over the past decade, possibly indicating a growing awareness of their significance.

This same group of researchers conducted a larger study involving 414 APS patients to investigate whether increasing physical activity reduces the risk of developing metabolic syndrome. They confirmed that physically active patients had a lower likelihood of developing metabolic syndrome (29).

Studies examining the effect of physical activity on cardiovascular risks in APS are limited. The beneficial impact of physical activity on preventing cardiovascular diseases and slowing the atherosclerotic process has been most extensively investigated in SLE patients. Legge et al. studied 100 SLE patients and found that just 10 minutes of light exercise reduced systolic and diastolic blood pressure as well as the 10-year risk of cardiovascular diseases. Increased physical activity led to more significant effects (30). Similarly, Tanaka et al. analyzed the influence of physical activity on arterial stiffness, measured through carotid-femoral pulse wave velocity (cfPWV), and found that arterial elasticity was higher in individuals engaged in moderate and substantial physical activity (31). A similar study conducted on Swedish subjects by Fernberg et al. yielded comparable results (32).

The low level of physical activity observed in PAPS patients can often be attributed to chronic fatigue and kinesiophobia. Bearne et al. emphasized that chronic fatigue is a significant limiting factor for achieving an adequate level of physical activity in this patient population, which can worsen their mental and physical condition and accelerate the progression of the primary disease (33). Bağlan Yentür and colleagues conducted a study on kinesiphobia and its impact on patients with SLE, highlighting that the fear of pain can deter movement and exacerbate atherosclerosis (34). Adequate treatment of kinesiphobia is thus crucial for the rehabilitation of these patients. A recent meta-analysis by Belgian authors included available studies on physical activity in SLE and demonstrated that appropriate physical activity significantly reduces chronic fatigue, emphasizing the need for more randomized studies to enhance rehabilitation strategies (35). Another meta-analysis by Blaess et al. underscored the issue of low physical activity levels in SLE patients and the favorable effects of aerobic training in this population, calling for clear recommendations (36).

LIMITATIONS

Our study has several limitations. Being a single-center study, its generalizability is restricted. The small sample size and the use of the IPAQ, which assesses physical activity only over the past week, pose challenges for drawing broader conclusions. Additionally, the cross-sectional design prevents the determination of causality or the assessment of longitudinal changes. Nevertheless, these limitations highlight the need for future research to validate and build upon our findings.

CONCLUSION

To the best of our knowledge, our study stands as the sole investigation to analyze the relationship between physical activity levels and the progression of the atherosclerotic process, as manifested by the prevalence of atherosclerotic plaques in the carotid arteries among APS patients. Beyond establishing a significant correlation between the level of physical activity measured in MET-min/week and the advanced stage of the atherosclerotic process, our study revealed that a significant proportion of APS patients, both primary and associated with SLE, exhibit low or moderate levels of physical activity. These findings underscore the need for clear guidelines and the promotion of physical activity within this patient population.

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Author contributions: AD, LS, SPD, and NS contributed to the conceptualization of the study and patient selection. AD and KB drafted the manuscript, while PM, RZ, MB, BF, and MP reviewed and revised the final version.

Ethical approval: The study obtained approval from the University Hospital Clinical Centre Bezanijska kosa ethical committee (7673/1/2021; date: 19.10.2021.).

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

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UTICAJ FIZIČKE AKTIVNOSTI NA PROGRESIJU ATEROSKLEROZE KOD BOLESNIKA SA ANTIFOSFOLIPIDNIM SINDROMOM

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Sažetak

Uvod: Antifosfolipidni sindrom (AFS) je povezan s povećanim rizikom od kardiovaskularnih bolesti usled interakcije tradicionalnih faktora rizika i antifosfolipidnih antitela na endotel. Cilj ove studije bio je analiza fizičke aktivnosti (FA) kod srpskih AFS bolesnika i njena povezanost s progresijom ateroskleroze.

Materijal i metode: Ispitan je 51 bolesnik sa AFS-om (29 sa primarnim AFS-om, PAFS, prosečne starosti 44.0 ± 11.5 godina, i 22 sa AFS-om udruženim sa SLE-om, sAFS prosečne starosti 48.4 ± 11.7 godina) korišćenjem IPAQ upitnika, prevedenog na srpski jezik. FA je analizirana kroz domene: slobodno vreme, kućne aktivnosti, posao i transport, i klasifikovana u nizak, umeren i visok nivo. Svim ispitanicima urađen je Doppler ultrazvučni pregled karotidnih arterija radi utvrđivanja prisustva plakova.

Rezultati: Ukupna FA u PAFS kretala se od 0 do 26.880 MET-min/nedeljno a u sAFS od 0 do 19.200, sa najvišim vrednostima za posao i kućne aktivnosti, a najnižim za slobodno vreme. Većina bolesnika imala je nizak ili umeren nivo FA (37,3% i 43,1%). Pacijenti s nižim skorom FA imali su značajno veću prevalencu karotidnih plakova, razlika je bila značajna za FA velikog intenziteta (p=0.001) i ukupnu FA (p=0.002).

Zaključak: Mladi AFS bolesnici u Srbiji imaju uglavnom nizak ili umeren nivo FA, s najnižim vrednostima u slobodnom vremenu. Nizak nivo FA povezan je s progresijom ateroskleroze, naglašavajući važnost promocije fizičke aktivnosti u ovoj populaciji.

Ključne reči: antifosfolipidni sindrom; ateroskleroza; fizička aktivnost;

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REVIEW ARTICLE

Importance of antithrombin evaluation and supplementation in clinical practice

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Summary

Antithrombin (AT) is a key natural anticoagulant that primarily inhibits thrombin, factor Xa, and other procoagulant proteases, maintaining hemostatic balance. Deficiency in AT, whether inherited or acquired, significantly increases the risk of venous thromboembolism, obstetric complications and sporadically arterial thrombosis. Individuals with AT deficiency are 15 times more likely to develop thrombosis.

Acquired deficiency is more common and may result from hypoproduction, excessive excretion or loss, increased consumption, or dilution of AT. In critically ill patients, acquired antithrombin deficiency may occur frequently; however, routine monitoring of antithrombin levels is not currently supported by strong evidence and should be reserved for selected clinical indications, especially with risk of thromboembolism. Antithrombin is essential for the action of unfractionated heparin, low-molecular-weight heparin, and fondaparinux, meaning deficiency can lead to resistance to these anticoagulants. Antithrombin deficiency is often detected in cases of resistance during the administration of antithrombin-dependent anticoagulants, typically indicated by insufficient levels of anti-Xa activity.

Supplementation of AT is indicated for preventing and treating thrombotic events in patients with congenital or acquired AT deficiencies.

Supratherapeutic antithrombin levels during heparin therapy can lead to bleeding. Its role in thromboembolism, anticoagulant resistance, and pleiotropic effects highlights its clinical importance and research potential.

Keywords: antithrombin, thromboembolism, heparin resistance, hemostasis

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INTRODUCTION

Antithrombin (AT) is an anticoagulant glycoprotein from the serpin superfamily, encoded by the SERPINCS-1 gene, which promotes the proteolytic activity of procoagulant proteases from both the extrinsic and intrinsic coagulation pathways. It represents the most potent natural inhibitor of coagulation, providing up to 80% of the potential to inhibit the formation of FIIa (thrombin). It also significantly inhibits FXa and, to a lesser extent, FIXa, FXIa, and FXIIa, thereby maintaining the fundamental balance of the hemostatic system (1,2).

In individuals with detected antithrombin deficiency, the risk of venous thromboembolism (VTE) is approximately 15 times higher. It is estimated that 50% of individuals with AT deficiency will experience a venous thromboembolic event before the age of fifty (3). About 8.8% of patients with AT deficiency and idiopathic VTE experience recurrent thromboembolic events annually (1,4).

CLINICAL PRESENTATION OF ANTITHROBIN DEFICIENCY

Antithrombin deficiency manifests through early onset of spontaneous (so-called idiopathic) venous thrombosis, with the fact that AT deficiency potentiates the formation of thrombosis in certain specific conditions (use of contraceptive therapy, pregnancy, trauma, immobilization, surgical interventions). AT deficiency can present as rare but potentially life-threatening forms of thromboembolism, such as cerebral venous sinus thrombosis, splenic vein thrombosis, Budd-Chiari syndrome, or thrombosis of the inferior vena cava. AT deficiency is also characterized by frequent recurrence of thrombosis. In addition to venous thromboembolism as the most common manifestation, antithrombin deficiency during pregnancy leads to certain obstetric complications, such as fetal growth restriction, placental abruption, fetal distress. It can also result in recurrent miscarriages and pregnancy loss. In rare cases, AT deficiency can present as arterial thrombosis, such as myocardial infarction. Congenital AT deficiency may be associated with a variety of other conditions and symptoms (developmental anomalies, psychomotor retardation, dysmorphic phenotype, microcephaly, small hands and feet, brachydactyly, clinodactyly of the fifth finger, facial dysmorphism, autoimmune symptoms, hypopituitarism, lipomatosis, skeletal and cardiac malformations, renal and hepatic insufficiency) (1).

The estimated prevalence of inherited antithrombin deficiency is between 1 in 2000 and 5000 individuals. It has been found that AT deficiency is detected in 2-3% of patients with thrombotic incidents. Given the clinical significance and the potentially life-threatening complications, it motivates physicians to always evaluate the presence of this type of thrombophilia. AT deficiency has been found in 4% of patients with recurrent VTE who are ≤ 50 years old, in 1% of patients with splenic vein thrombosis, and in 2% of cases associated with the use of combined oral contraceptives or pregnancy (1,5, 6).

SUBTYPES OF ANTITHROMBIN DEFICIENCY

Inherited Antithrombin Deficiency

Inherited Type I antithrombin deficiency results from reduced synthesis or stability of antithrombin due to specific mutations. It is characterized by a proportional decrease in both the concentration and functional activity of antithrombin, with an antigen/activity ratio of approximately 1. In contrast, inherited Type II deficiency is characterized by the production of altered, nonfunctional antithrombin due to mutations that lead to a lack of functional activity. In this case, the level of antithrombin in the blood is normal, but the antigen/activity ratio is greater than 1 **(Table 1)** (2).

Inherited Type II antithrombin deficiency is further divided into three subtypes. Subtype IIa or Type II Reactive Site (II RS) is characterized by a reduced ability of antithrombin to bind to proteases like thrombin. Subtype IIb or Type II Heparin-Binding Site (II HBS) refers to a reduced ability of antithrombin to bind heparin, thereby diminishing its anticoagulant effect. Subtype IIc or Type II with Pleiotropic Effects (II PE) involves a structural defect in antithrombin near the thrombin-binding site. Individuals with Type IIc antithrombin deficiency usually have lower antithrombin levels compared to other subtypes. Subtypes IIa and IIc are associated with a more severe clinical picture of antithrombin deficiency, while individuals with subtype IIb deficiency have a lower risk of venous thromboembolism but a higher risk of arterial thromboembolism (3).

All inherited types of antithrombin deficiency occur in a heterozygous genotype, except for subtype IIb, which

Table 1. Types of inherited antithrombin deficiency

Types of inherited antithrombin deficiency	Disorder Characteristics	Genotype	Ag/Ac Ratio
Type I	Reduced AT concentration	Heterozygous	Approximately 1
Type IIa - *type II reactive site (II RS)*	Reduced AT binding to thrombin	Heterozygous	>1
Type IIb - *type II heparin-binding site (II HBS)*	Reduced AT binding to heparin	Heterozygous / homozygous	>1
Type IIc - *type II with pleiotropic effects (II PE)*	Multifunctional AT disorder	Heterozygous	>1

AT – antithrombin, IIRS - type II reactive site, IIHBS - type II heparin-binding site, IIPE - type II with pleiotropic effects

can be compatible with life even in a homozygous form. It is believed that Type II antithrombin deficiency is more common in the general population and carries a lower risk of thromboembolic events, except for subtype IIa, which carries a high risk for thromboembolic events. In contrast, individuals with symptomatic thrombophilia are more frequently diagnosed with Type I antithrombin deficiency (3).

Acquired Antithrombin Deficiency

Acquired antithrombin deficiency is significantly more common and is caused by: 1. Hypoproduction of antithrombin, such as in cases of acute and chronic liver insufficiency (e.g., liver cirrhosis), malnutrition, individuals undergoing L-asparaginase therapy, or premature infants. 2. Excessive excretion or loss of antithrombin, as seen in nephrotic syndrome, burns, inflammatory bowel diseases, and other enteropathies accompanied by protein loss. 3.Increased consumption, such as in disseminated intravascular coagulation (DIC), microangiopathy with thrombosis, malignancies, hematologic transfusion reactions, major surgeries (notably, the lowest levels of antithrombin are recorded on the third postoperative day), heparin therapy (after 4-5 days of heparin infusion antithrombin levels can be reduced to 50-60% of normal), massive thrombosis, severe sepsis, multiple trauma, hemolytic-uremic syndrome (HUS), preeclampsia, eclampsia. 4. Dilution, during massive transfusions, use of ECMO (extracorporeal membrane oxygenation), extensive use of intraoperative blood salvage devices, and extracorporeal circulation methods. 5. Complex and combined mechanisms, such as in sepsis, where there is a simultaneous process of decreased production and increased consumption, or in conditions like preeclampsia, eclampsia, and HELLP syndrome, which include reduced production and increased consumption of AT in combination with endothelial dysfunction **(Table 2)** (2,7).

Antithrombin levels lower than 50-60% in sepsis are generally associated with poorer prognosis, while levels below 20% correlate with fatal outcomes. In acquired forms of antithrombin deficiency, other anticoagulant proteins like protein C and protein S are often reduced as well (1). Postoperative chylothorax in children is associated with an increased risk of vascular thrombosis, believed to result from the loss of antithrombin in chyle fluid, leading to a hypercoagulable state (8).

It is considered that in patients with Budd-Chiari syndrome, there are other causes of thrombosis in addition to antithrombin deficiency (8). The use of oral contraceptives and estrogens can, in certain predisposed individuals, lead to antithrombin deficiency (1,9).

Ovarian hyperstimulation syndrome (OHSS) is a severe complication that can occur in women undergoing controlled ovarian stimulation during in vitro fertilization (IVF). The estimated incidence of this syndrome is between 1%-5%, but it can reach 10% in individuals with risk factors (10). The clinical presentation of OHSS ranges from mild symptoms to potentially life-threatening thromboembolic complications. Arterial events predominantly involve cerebrovascular accidents, which typically occur at the onset of OHSS. Venous thromboses occur several weeks later and are often reported in unusual but specific locations, such as the large veins of the upper extremities and neck (11). According to current knowledge, hypercoagulability in OHSS is contributed to by vasoactive substances from the ovaries, hemoconcentration, hypovolemia leading to arterial hypotension, elevated 17β-estradiol levels due to induced ovulation, and the administration of human chorionic gonadotropin (hCG). Inherited thrombophilia and antithrombin deficiency can also contribute to the development of OHSS (7). On the other hand, studies indicate that hormonal stimulation with human chorionic gonadotropin can lead to a decrease in antithrombin concentration (11).

DIAGNOSIS OF ANTITHROMBIN DEFICIENCY

Evaluating antithrombin (AT) levels in the blood can help define the etiopathogenetic substrate of thromboembolic disease, enable substitution therapy, and significantly influence the outcome of the disease.AT activity levels between 70-80% are considered borderline and indicate a potential AT deficiency, while levels below 70% are considered diagnostic (12). The majority of patients with AT deficiency are detected by reduced anti-Xa levels during heparin derivatives therapy, but some patients with

Table 2. Causes of acquired antithrombiin deficiency

hypoproduction	acute and chronic liver insufficiency (e.g., liver cirrhosis), malnutrition, L-asparaginase therapy, in premature infants.
extreme excretion or loss of AT	nephrotic syndrome, burns, inflammatory bowel diseases, and other enteropathies associated with protein loss
increased consumption	disseminated intravascular coagulation, microangiopathy with thrombosis, malignancies, hematologic transfusion reactions, major surgeries, heparin therapy, massive thrombosis, severe sepsis, multiple traumas, hemolytic-uremic syndrome,
dilution	massive transfusions, use of extracorporeal membrane oxygenation, extensive use of intraoperative blood salvage devices, extracorporeal circulation methods
complex and combined mechanisms	sepsis, preeclampsia, eclampsia, *HELLP* syndrome, *OHSS*

AT – antithrombin, HELLP syndrome – hemolysis, elevated liver enzymes, low platelet count syndrome, OHSS – ovarian hyperstimulation syndrome

pathogenic mutations may not be diagnosed using this test, in which case molecular diagnostics is advised (1). When interpreting blood antithrombin levels, special attention should be paid to the fact that the use of AT analysis while on direct oral anticoagulants (dabigatran, rivaroxaban, apixaban, edoxaban) can lead to falsely high AT levels, masking the diagnosis of AT deficiency. Additionally, bilirubin levels above 50 mg/dL, hypertriglyceridemia, and rheumatoid factor levels greater than 800 IU/mL can affect the AT level. Genetic testing can help identify patients with AT deficiency that standard tests may miss (13). To ensure accurate AT measurements, it is crucial to properly store samples. Freezer models with self-defrosting cycles may cause temperature fluctuations that can affect protein stability and test results. Therefore, freezers without automatic defrosting or ultralow-temperature freezers maintaining a constant temperature of -70°C are recommended (14).

Diagnostic algorithm in AT deficiency is presented in **Figure 1**.

THERAPEUTIC APPLICATION

In cases of significant antithrombin deficiency, AT substitution is performed using synthetic AT preparation. One of the formulas used to determine the required dose of AT is:

Dose (units required) = (120% - baseline %) × body weight (kg)/ 1.4 (2,3).

The use of antithrombin is indicated for the prevention and treatment of thrombotic events in individuals with either congenital or acquired AT deficiency. In general, therapeutic approaches for patients with AT deficiency are considered in three groups:

1. Treatment of acute thromboembolic events
2. Short-term prophylaxis when exposed to high-risk situations
3. Long-term prophylaxis for symptomatic patients who have had a previously documented episode of thromboembolism caused by AT deficiency (3).

Antithrombin is essential for the action of unfractionated heparin, low-molecular-weight heparin, and fondaparinux (anticoagulants dependent on antithrombin), which are first-choice drugs in the treatment of VTE. In cases of patients with AT deficiency, resistance to these anticoagulants can develop (3).

Antithrombin deficiency is the most common cause of true heparin resistance, characterized by aPTT and anti-Xa activity levels measured during heparin therapy that are concordantly lower than expected, as opposed to pseudo-heparin resistance, where aPTT is lower than expected, but anti-Xa activity is appropriately reduced with prescribed heparin therapy. Predictors of heparin resis-

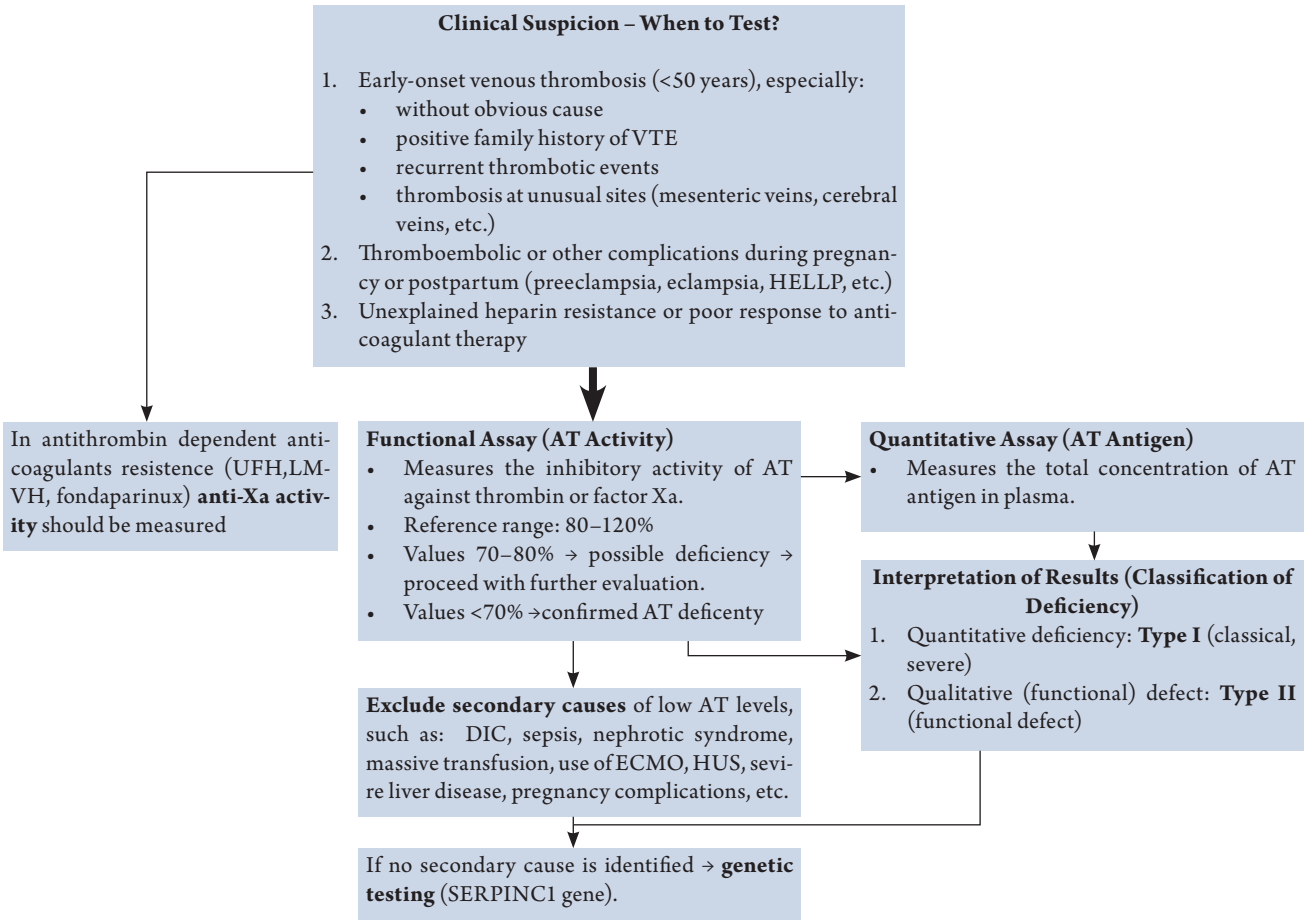

Figure 1. Diagnostic algorithm in AT deficiency

tance include AT activity ≤ 60%, platelet count >300 × 10^9/L, age (>65 years), and elevated levels of factor VIII and fibrinogen (15).

Optimizing heparin therapy based only on aPTT may lead to overdose and hemorrhagic events in cases of pseudo-heparin resistance, often caused by elevated FVIII levels and other acute-phase reactants (16). The laboratory-detected anti-Xa activity level, measured to optimize heparin therapy, does not depend on acute-phase reactants, unlike aPTT (16).

Heparin resistance is effectively resolved by AT supplementation, with preference given to AT concentrate (ATc). At the same time, in modern clinical practice, fresh frozen plasma is not recommended except when ATc is unavailable due to potential risks like allergic reactions, circulatory overload, infection transmission, hemolytic transfusion reactions, alloimmunization, and transfusion-related acute lung injury (TRALI) (3).

Long-term prophylaxis for symptomatic patients with AT deficiency involves the use of warfarin or direct oral anticoagulants (DOAC), which are AT-independent (3, 17, 18).

During pregnancy, especially in the postpartum period, women with AT deficiency are at high risk for developing VTE. Modern guidelines recommend pharmacological thromboprophylaxis with low-molecular-weight heparin in all women with mild AT deficiency during pregnancy and postpartum. AT substitution may also be considered for pregnant women and women in the postpartum period as adjunct therapy in high-risk cases or those with significantly reduced AT levels (19).

Drug overdose, determined by antithrombin levels exceeding 120%, can cause a hemorrhagic tendency and lead to manifest bleeding, especially when AT preparations are given alongside heparin (1). If AT levels exceed 120%, it is recommended to reduce the dose by 30%. If the level is below 80%, the dose should be increased by 30%, with AT levels checked 2 hours after infusion completion. Before administration of the next dose, AT levels should be checked in order to optimize therapy. Patients with prior thrombosis and AT deficiency, should be considered for prolonged or lifelong anticoagulant therapy (1,2).

A specific problem for patients with undiagnosed AT deficiency is the use of low-molecular-weight heparin for anticoagulation therapy, whose therapeutic effect is rarely monitored by anti-Xa activity levels. Failure to achieve the target therapeutic effect with antithrombin-dependent anticoagulants prescribed for thrombosis prevention or treatment can endanger the patient's life. Clinicians should also be aware that heparin resistance can be caused by nitroglycerin infusion and the use of antidotes for direct anticoagulants, such as andexanet alfa (1,2,20,21).

In addition to its role as an anticoagulant protein and essential factor for the action of antithrombin-dependent anticoagulants, AT also may possess anti-inflammatory, anti-angiogenic, antimicrobial, and even antiplatelet properties, exerted through the release of prostacyclin from endothelial cells (1,2,20).

The mechanism of the anti-inflammatory response likely lies in the immunomodulatory effect of AT binding to heparan sulfate proteoglycans on the surface of endothelial cells, reducing the production of inflammatory and procoagulant mediators, while increasing the release of anticoagulant prostacyclins (PGI) (22).

Given the anticoagulant and anti-inflammatory effects of AT, its substitution may potentially benefit the treatment of individuals with sepsis-induced disseminated intravascular coagulation (DIC), sinusoidal obstruction syndrome, previously known as veno-occlusive liver disease, for patients undergoing ECMO therapy, therapy of acute lymphoblastic leukemia with asparaginase, and perioperative preparation of patients undergoing cardiopulmonary bypass. These indications for AT substitution are still under investigation (3).

Studies have shown that some cases of disseminated intravascular coagulation (DIC) associated with antithrombin deficiency (consumptive coagulopathy develops accompanied by low anticoagulant and fibrinolytic activity), present with the development of a severe prothrombotic state with significantly worse prognosis. In such cases, in addition to assessing the SOFA and DIC scores, monitoring antithrombin (AT) levels is recommended both for evaluating the severity and prognosis of the disease, as well as for selecting appropriate therapy (23).

Some observation studies have shown improved treatment outcomes in sepsis-induced disseminated intravascular coagulopathy patients associated with AT deficiency; however, most global protocols and guidelines call for further research. Only in Japan's current guidelines is AT substitution approved for this indication (24,25).

There are some indicators of potential benefit from the use of AT in various malignancies, such as glioblastoma, lung cancer, and colorectal cancer, due to its observed anti-angiogenic effect, reduced tumor protein expression, and suppression of metastatic spread (3).

Routine substitution of antithrombin in critically ill patients is not standard practice. It is recommended only in cases of documented deficiency with a clear clinical indication, such as heparin resistance, planned invasive procedures, or active thrombosis.

The use of AT concentrates in patients with sepsis and disseminated intravascular coagulation (DIC) remains controversial, as clinical studies have shown inconsistent results. Many of these studies are methodologically limited, with heterogeneous primary outcomes, non-standardized dosing, and a lack of well-designed randomized controlled trials (26, 27, 28).

CONCLUSION

AT deficiency often goes unrecognized in everyday clinical practice, so more frequent evaluation is necessary. It is essential to evaluate the level of AT in order to define the exact pathogenetic mechanism of thrombotic events, especially major unprovoked thromboembolic episodes in a specific population. Although AT deficiency is commonly detected during heparin therapy and suspected of causing heparin resistance, it is crucial to recognize acquired conditions that carry a risk of AT deficiency. Regulating AT levels is critically important in the prophylaxis and therapy of thrombotic events. Although AT activity showed a strong positive correlation with higher heparin requirements, higher average AT activity did not show a significant difference in the rate of bleeding events. Therefore, future studies should compare the effects of higher endogenous AT activity with exogenously supplemented AT activity to differentiate between their contributions to the survival benefit.

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ZNAČAJ EVALUACIJE I SUPLEMENTACIJE ANTITROMBINA U KLINIČKOJ PRAKSI

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Sažetak

Antitrombin (AT) je ključni prirodni antikoagulans koji inhibira trombin (FIIa), faktor Xa (FXa), u manjoj meri i druge prokoagulantne proteaze, održavajući hemostatski balans.

Nedostatak AT, nasledni ili stečeni, značajno povećava rizik za nastanak venskog tromboembolizma (VTE), različitih opstetričkih komplikacija, a kod određenih subtipova i arterijskih tromboza. Osobe sa deficitom AT imaju 15 puta veći rizik za razvoj tromboza.

Stečeni deficit antitrombina nastaje usled hipoprodukcije, gubitka, povećane potrošnje, dilucijom ili kombinacijom navedenih patoloških procesa i dosta je češći u odnosu na urođeni deficit AT.

Kod kritično obolelih pacijenata često se detektuje stečena deficijencija antitrombina; međutim, rutinsko praćenje nivoa antitrombina trenutno nije podržano snažnim dokazima i trebalo bi da se sprovodi samo u odabranim kliničkim indikacijama posebno kod onih sa visokim rizikom od tromboembolije.

Antitrombin je neophodan za dejstvo nefrakcionisanog heparina, niskomolekulskog heparina i fondaparinuksa pa u slučaju njegovog nedostatka dolazi do razvoja rezistencije na dejstvo navedenih lekova. Deficit AT se često detektuje prilikom nalaza rezistencije na primenu gore pomenutih od antitrombina zavisnih antikoagulanasa kada se registruje insuficijentni nivo anti Xa aktivnosti.

Primena antitrombina indikovana je u prevenciji i terapiji trombotičnih događaja kod osoba sa urođenim ili stečenim deficitom.

Malo je poznato da supraterapijski nivoi antitrombina uz heparinsku terapiju mogu izazvati hemoragijsku tendenciju i dovesti do manifestnih krvarenja. Uloga AT u patogenezi tromboembolizma, rezistenciji na antikoagulanse, i njegova plejotropna dejstva upućuju na veliki klinički značaj i potencijal za dalja istraživanja.

Ključne reči: antitrombin, tromboembolizam, heparinska rezistencija, hemostaza

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REVIEW ARTICLE

Treatment of dyslipidemia: PCSK-9 in focus

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Summary

Cardiovascular disease is the leading cause of death worldwide, accounting for one-third of the total global mortality. Dyslipidemia is one of the most common risk factors and plays a cardinal role in the development and progression of atherosclerotic cardiovascular disease. Since statin therapy is often insufficient, or cardiovascular disease continues to develop despite achieving target lipid levels, current attention is focused on new therapeutic options with significantly greater efficacy. The reduction in LDL cholesterol levels with PCSK-9 inhibitors is about 60% when used as monotherapy and as much as 85% when combined with high-intensity statins and/or other lipid-lowering therapies. Alirocumab and evolocumab are two monoclonal antibodies that effectively bind to the LDL receptor, interfering with its degradation. Inclisiran is a small interfering RNA that interferes with the synthesis of PCSK-9 molecules and effectively reduces LDL-C. Indications for PCSK-9 inhibitors usage are increasingly expanding due to the discovery of their pleiotropic effects. Additionally, the focus is on discovering new mechanisms of PCSK-9 inhibition that would open the door to the development of new therapeutic agents. Studies on the efficacy and safety of oral PCSK9 inhibitors are ongoing, along with early-stage clinical trials exploring gene-editing strategies for the treatment of dyslipidemias.

The purpose of this review article is to provide an overview of the current knowledge on the application of PCSK-9 inhibitors.

Keywords: dyslipidemia, LDL cholesterol, PCSK-9

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for one-third of all-cause mortality. It is estimated to claim around 17.9 million lives each year (1,2). It was the Framingham heart study of the mid-20th century that first established a causative relationship between lipids and accelerated atherogenesis and atherosclerotic cardiovascular disease (ASCVD) (3). Risk factors for ASCVD include hypertension, diabetes, dyslipidemia, tobacco smoking, lack of physical activity and sedentary lifestyle (4,5). Dyslipidemia is the most prevalent risk factor for CVD, playing a crucial role in the onset and advancement of ASCVD (2-4). Dyslipidemia represents an imbalance of lipid metabolism manifested by elevated values of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and triglycerides (Tg) and/or decreased values of high-density lipoprotein-cholesterol (HDL-C) or a combination of mentioned disorders (5-7). The USA data indicate that more than 30% of adults have elevated TC and LDL-C, while approximately 20% have elevated Tg values (7). According to WHO data, dyslipidemia is the cause of more than 4 million deaths each year worldwide (8).

Dyslipidemia can be classified as a primary (inherited) disorder of gene mutation causing overproduction or defective clearance of lipid fractions or as a secondary disorder because of other medical conditions or medication use (6,9,10). Data from 2019 suggest that increased values of LDL-C are responsible for 44% of coronary artery disease (CAD) mortality and 22% ischemic stroke mortality (10). Inherent dyslipidemias can be heterozygous or homozygous. Individuals with heterozygous familial hypercholesterolemia (FH) in most WHO regions are noted to have a higher prevalence of type 2 diabetes compared to those in Europe. Obesity significantly elevates the risk of diabetes in these individuals, particularly when associated with age and use of FH lowering medications (11). Lifestyle interventions focusing on diet and physical activity have been widely studied for their role in reducing obesity, and have shown promising outcomes not only in the prevention of type 2 diabetes (T2D) but also in lowering the risk of cardiovascular diseases (CVD) (12).

In regards of CVD risk assessment, the SCORE (Systemic Coronary Risk Estimation) calculator is in use, particularly the SCORE-2 for estimating the 10-year CVD risk and SCORE2-OP for estimating CVD risk in older individuals (13). However, despite effective risk assessments and the use of standard therapeutic strategies new cardiovascular (CV) events still occur. Identifying FH patients and other high-risk individuals is a key priority in modern preventive cardiology and clinical lipidology (14). It has become evident that residual CVD risk remains implementation of standard medical treatments and interventions, even after achieving LDL-C target values (15,16). Recently, some of the new studies have suggested that the levels of LDL-C are not the only factors significant for the development of ASCVD, but also the cumulative exposure to elevated cholesterol levels over the years, suggesting the necessity of using drugs as early as possible to lower LDL-C levels and reduce the risk for ASCVD (17). Clinical trial results with newly developed lipid-lowering agents demonstrated a significant decrease in CV events. These agents' mechanism of action includes enhancing LDL receptor (LDLR) expression or reducing cholesterol absorption, leading to a substantial reduction in LDL-C (18). The focus has thus shifted from "high-intensity statin therapy" to "high-intensity LDL-C lowering" incorporating combination therapy at an early stage (19). This is how the concept of "lower is better" was born (20). Guidelines emphasize the need for early detection and increased utilization of combination therapies to mitigate the global burden of familial hypercholesterolemia (21).

GUIDELINES FOR MANAGING DYSLIPIDEMIAS: FOCUS ON LIPID MODIFICATION TO REDUCE CV RISK AND OUTLINE THE ROLE OF PCSK-9 INHIBITORS

The most recent comprehensive guidelines of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) for the treatment of dyslipidemia recommend that, after precise CV risk stratification, stepwise approach should be applied, starting with statins, then adding ezetimibe and finally proprotein-convertase subtilisin/kexin type-9 (PCSK-9) inhibitors **(Table 1)** (22,23). The use of high intensity statins is recommended as the first-line therapy for primary and secondary prevention in patients with hypercholesterolemia. If LDL-C target values are not reached for patients on the maximum tolerated dose of statins, adding ezetimibe is recommended as a second-line treatment for primary and secondary prevention. If the desired targets are not reached with the aforementioned combination therapy (statin+ezetimibe), the addition of a PCSK-9 inhibitor is advised in secondary prevention (evidence class Ia).

Furthermore, the guidelines additionally state that PCSK-9 inhibitors can also be recommended for primary CV prevention in high-risk FH patients (with ASCVD or with other major risk factor) who did not reach LDL-C target levels despite maximum tolerated statin therapy and ezetimibe (evidence class Ic). Also, ezetimibe and/or PCSK-9 inhibitors should be considered in patients with dyslipidemia who have statin intolerance as an addition to ezetimibe (evidence class IIb/C) (22,23). Having in mind that early and effective lowering of LDL-C is necessary for a better reduction of new CV events, a new consensus statement highlights the need to adopt combination lipid-lowering therapy as first-line strategy for very-high risk patients, including combination with PCSK-9 inhibitors (19).

Table1. Recommendation for dyslipidemia treatment (The submitted table is the work of the author and is based on information available in the cited reference European Heart Journal, Vol 41, Issue 1, 2020, 111–188.)

CVrisk cegory	Risk factors	Target levels of plasma LDL-C and therapy approach
Low risk	• SCORE<1%	LDL-C<3.0 mmol/L
Moderate risk	• SCORE 1-5% • Young patients (T1DM <35 years, T2DM<50 years without other RF)	LDL-C<2.6 mmol/L
High risk	• 10-year SCORE risk ≥ 5% and ≤10% • Patients with total-C > 8 mmol/l, LDL>4.9 mmol/l or BP≥180/110 mmHg • Patients with diabetes without complications, if the disease lasts longer than 10 years with other risk factors • Moderate HBI (GFR 30- 59mL/min/1.73m^2) • FH without risk factors	LDL-C<1.8 mmol/L **Moderately intense statin**
Very high risk	• 10-year SCORE risk ≥ 10% • Documented ASKVB - clinical methods or visualization methods • Type 2 diabetes with macro and microvascular complications or with 3 risk factors present • Type 1 diabetes with micro and macrovascular complications, or with 3 risk factors present or if the disease lasts longer than 20 years • Severe HBI (GFR <30mL/min/1.73m^2) • FH with ASKVB or with risk factors	LDL-C<1.4mmol/L or <50% reduction in LDL-C if the goal is not reached Application of high-intensity therapy for LDL reduction **Maximum dose of statin+ezetimibe+ PCSK9 inhibitor**
Extremely high risk	• Patients with ACS who experience a new cardiovascular event within two years (does not have to be of the same nature as the first) and they are already getting maximally tolerable dose of statin	LDL-C< 1.0 mmol/L Application of high intensity LDL-C reduction therapy **Maximum dose of statin+ezetimibe+PCSK9 inhibitor**

Other therapeutic agents for dyslipidemia treatment are also in use. Bempedoic acid has been approved for use in patients who are statin intolerant. The main therapeutic objective in treating dyslipidemias is to reduce LDL-C, however there is now clear evidence that lowering lipoprotein Lp (a) levels also help reduce CVD risk as it is also a standalone risk factor for CVD additionally (23). Pelacarsen and olpasiran are emerging therapeutic options designed at lowering Lp (a). LDL apheresis is a specialized medical treatment, like dialysis, for lowering LDL-C. It is typically intended for patients with severe hypercholesterolemia that are unresponsive to conventional treatment or for individuals with statin intolerance or high Lp (a) cholesterol (24,25). Other innovative small molecules such as evinacumab, lomitapide, mipomersen, volanesorsen and olezarsen target different stages in the metabolism of atherogenic lipoproteins, and show great promise in FH therapy (2,13,20).

PCSK-9 INHIBITORS AS A NEW THERAPEUTIC AGENT FOR LOWERING LDL-C

Discovery of PCSK-9

The Nobel Prize awarded to Goldstein and Brown was a landmark discovery of the role of the LDLR, which opened the door to further research that brought new discoveries including that of PCSK-9. This new knowledge and understanding of LDLR function in clearing LDL-C, explained the mechanism of FH that lay in LDLR gene mutation. Additionally, novel cellular processes such as receptor-mediated endocytosis, receptor recycling and receptor regulation have been described. Later, a major regulatory pathway was discovered whose role is to control the number and function of LDLR. It was then that the PCSK-9 enzyme and its gene on chromosome 1 were identified for the first time (26). The physiological role of PCSK-9 was first recognized through the discovery that functional mutations in the PCSK-9 gene lead to dominant FH. A 2003 study identified a mutation in the PCSK-9 gene in French families with no known mutations in the LDLR or apolipoprotein B (APOB) genes, which was linked to markedly elevated concentrations of LDL-C (27). A gain-of-function (GOF) mutations in the PCSK-9 gene indicated that there was a new factor in cholesterol homeostasis still unknown. The Dallas Heart Study, conducted on a multi-ethnic population including a subset of African Americans found a loss of function mutation (LOF) in the PCSK-9 gene, linked to reduced serum LDL-C and a significantly lower incidence of CVD was observed (28). These findings put a spotlight on PCSK-9 which in short time become a promising treatment target for FH.

Role of PCSK-9 on lipid metabolism

PCSK-9 is an enzyme predominantly synthetized in the liver. Its function is reflected in its binding to the LDLR which is then degraded at the lysosome, resulting in the

reduction of LDL receptor recycling, downregulating receptor activity. The main consequence is the increase of LDL-C concentration in the serum (28-30). When not bound to PCSK-9, LDLR binds to LDL-C and enters the cell, separates from LDL-C in the endosomes and is returned to the plasma membrane, while LDL-C is directed to lysosomes for breakdown (31). PCSK-9 gene variants account for 2–4% of FH. These gene mutations cause grater cholesterol concentration than LDLR or APOB polymorphisms (32). Patients with GOF mutations are predisposed to elevated LDL-C levels and the development of FH accompanied by an increased CV risk with cholesterol levels exceeding 13 mmol/L (33). Individuals with LOF mutations in PCSK-9 exhibit an increased density of liver LDLR accompanied by decreased LDL-C concentrations, resulting in CVD reduction of 50 to 86% (34).

In recent years an idea of blocking PCSK-9 with a monoclonal antibody has first come to light. Inhibition of the PCSK-9 enzyme with monoclonal antibodies increases the availability of LDLR ("upregulation of the LDLR") and enhancing LDL-C clearance. Effectiveness of PCSK-9 inhibitors depend on the presence of LDLR, in that sense these medications are ineffective in patients with homozygous FH (HoFH) (28). To date, two fully human monoclonal antibodies are available: alirocumab and evolocumab. Both antibodies are developed using transgenic mouse platforms and work by preventing the interaction between PCSK-9 and LDLR. Alirocumab and evolocumab can reduce LDL-C levels by 60–70% and are approved for the treatment of patients with FH and established CVD (35). In addition, a novel strategy utilizing RNA interference therapy has been developed to inhibit the cellular production of PCSK-9. Inclisiran, a novel molecule, is a synthetically produced small interfering RNA (siRNA) that targets PCSK-9 **(Figure 1)**. This small interfering RNA attaches within the cell an RNA-induced silencing complex (RISC), which in turn cuts mRNA molecules responsible for encoding PCSK-9. The fragmented mRNA is broken down through a catalytic process to prevent PCSK-9 protein synthesis in the liver. Inclisiran's RNA–induced silencing complex can degrade multiple PCSK-9 mRNAs (36). Within 24 hours of subcutaneous injection, inclisiran becomes undetectable in plasma due to its rapid, specific, and efficient uptake by hepatocyte (31). Recent studies have shown that the molecule resistin-adipokine from adipose tissue lowers the expression of the LDLR at the level of hepatocytes subsequently increasing the expression of PCSK-9 molecule. This fact potentially opens the possibility for new therapeutic options that would aim at the resistin-adipokine inhibition resulting in ASCVD prevention (26).

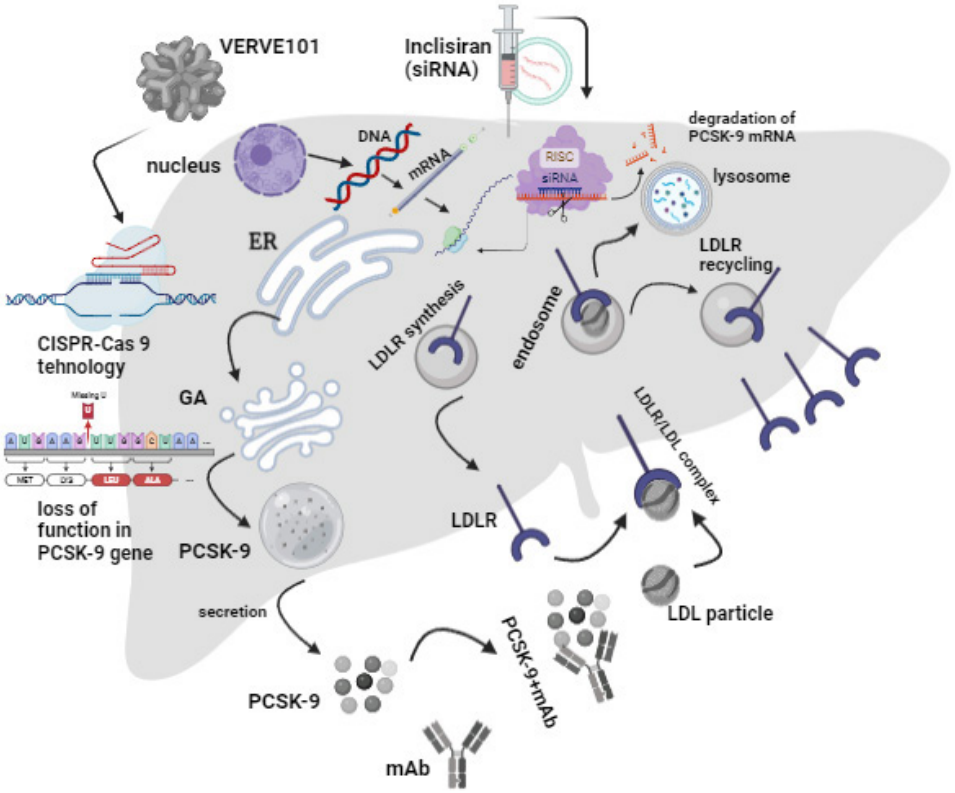

Figure 1. Mode of action of PCSK-9 inhibitors (The submitted figure is the work of the author and is based on information available in the cited references European Heart Journal 2022;43(34):3198-3208)

PCSK-9 - proprotein-convertase subtilisin/kexin type-9; mAb – monoclonal antibody; CISPR – Cas 9 - clustered regularly interspaced short palindromic repeats; LDL - low density lipoprotein; LDLR- low density lipoprotein receptor; RISC - RNA-induced silencing complex; ER – endoplasmatic reticulum; GA – Golgi apparat; mRNA - messenger RNA, DNA - deoxyribonucleic acid.

Additional benefits of PCSK-9 inhibitors

It has been observed that PCSK-9 inhibitors have a strong influence in reducing CV risk besides solely reducing LDL-C, or rather, PCSK-9 inhibitors have other pleiotropic effects that make them extremely superior (26). It is unequivocally confirmed that PCSK-9 inhibitors decrease the concentrations of pro-inflammatory cytokines while promoting the production of anti-inflammatory cytokines (e.g., IL-10). Additionally, their use reduces the expression of TNF alpha and C-C chemokine receptor type 2 (CCR2). As mentioned earlier, PCSK-9 inhibitors reduce oxidative stress by lowering the production of NADPH oxidase and the production of adhesive membrane molecules (ICAM and VCAM) (37,38). In addition, PCSK9 inhibition ultimately contributes to the stabilization of atherosclerotic plaques by reducing the necrotic core of the atheroma, as demonstrated through intracoronary imaging in randomized controlled trials such as PACMAN-AMI, HUYGENS, and GLAGOV (37). The studies have also shown that PCSK-9 inhibitors in some degree have a direct proinflammatory effect on blood vessels, through LDLR related protein 1 (LDL-RP1). All these mechanisms are key pathophysiological factors of the evolution of ASCVD (37-40).

PCSK-9 inhibitors are associated with both antiaggregant and anticoagulant effects through direct and indirect activation of the scavenger receptor CD36 and low-density lipoprotein receptor-1 (LOKS-1) on the hepatocyte membrane. They also exabit an antiplatelet effect by lowering Lp(a) levels, which reduces the stimulation of toll-like receptor 2 (TLR2) on the surface of lipid-peroxide-modified phospholipids, transported specifically by Lp(a) particles. The anticoagulant effect is reflected in the reduction of tissue factor and factor VIII levels (37,41).

There is data that PCSK-9 inhibitors also have an antineoplastic effect by reducing serum levels of LDL-ch and TG. Reduction of PCSK9 activity has a function in inhibiting the progression of colorectal cancer and breast cancer (42). Their effect on TLR and their modulation could have a significant function in regulating the immune response to sepsis and septic shock (43).

PCSK-9 inhibition by monoclonal antibodies: evolocumab and alirocumab

Evolocumab and alirocumab are anti-PCSK9 monoclonal antibodies approved for clinical use, either as monotherapy or in combination with statins and/or ezetimibe. These monoclonal antibodies reduce LDL-C levels around 60% in monotherapy and even 85% together with other hypolipemic therapies. They can be administered every two weeks or once a month. FOURIER and ODYSSEY are randomized, double-blind, placebo-controlled trials that demonstrated that adding evolocumab or alirocumab to standard lipid-lowering therapy in high cardiovascular risk patients significantly reduces the absolute risk of ischemic events and overall mortality (29,30).

The FOURIER study demonstrated that Evolocumab reduces LDL-C levels in plasma by 53% to 75%, based on its use as monotherapy or in conjunction with statins in patients with heterozygous familial hypercholesterolemia (HeFH), while in HoFH with defective LDL-R, that percentage is drastically lower and amounts to about 31% (43). The incidence of cardiovascular death, myocardial infarction (MI), stroke, hospitalization for unstable angina, or coronary revascularization as the primary outcome was significantly lower in a statistical sense compared to the placebo group receiving statin therapy alone (13,30). Perhaps the most significant finding was that the benefit of using PCSK-9 was greater among high-risk subgroups with recent or multiple MI and multivessel coronary artery disease, in that regard PCSK-9 was recommended in guidelines for the treatment of dyslipidemia in secondary prevention (30,44). Dosage of evolocumab is 140 mg/ml every two weeks or 420 mg once a month (44).

The ODYSSEY study demonstrated that Alirocumab lowers LDL-C by 39% to 58% in patients with HeFH and by 11.9% to 34.3% in patients with HoFH (44). The study revealed that patients with recent CVD on high-intensity statin therapy had a significantly reduced risk of recurring cardiovascular events compared to the placebo group (45). Furthermore, it has been demonstrated that the use of Alirocumab has beneficial impacts in reducing oxidative stress, creation of inflammatory cytokines, lowering the function of metalloproteinase 2, osteopontin and osteoprotegerin, which are critical stages in the formation of atherosclerotic plaques (46). The dose of alirocumab is 75 mg once every two weeks with a possible titration up to 150 mg based on the level of LDL-C. Alternative to this dosing regimen is 300 mg once a month (44).

Besides lowering LDL-C, monoclonal antibodies reduce TC, Tg, non-HDL-C, apo B and slight increase in HDL-C. Unlike statin therapy, both available monoclonal antibodies can reduce an Lp(a) for up to 30% (44,45). There is no need to adjust the dose depending on age, gender, body weight, renal or mild to moderate hepatic insufficiency. In patients on previous statin therapy, the dose interval may be shorter because of the increased expression of the PCSK-9 molecule caused by statins. For now, there is no precise data for patients who have severe renal and hepatic insufficiency (47,48).

Two more trials, the EMACS and EVACS trials have directly demonstrated PCSK-9 superiority in combination with a statin in lowering LDL-C after acute coronary syndrome during hospital treatment, however, there is still no precise data on cardiovascular outcomes and serious side effects (49,50). The use of monoclonal antibodies has few side effects. With therapies targeting RNA symptoms of immune activation are always in focus but found to be rear in patients using inlisiran. Some flu-like symptoms were reported. Apart some mild injection-site

reactions, some neurocognitive events were recorded in patients taking monoclonal antibodies including dementia, delirium, cognitive disorders, amnestic and attention disorders, disturbances in thinking and perception, and mental impairment disorders (51). Alirocumab was evaluated in the ODYSSEY long-term trial, and the data indicated that it caused myalgia and injection site reactions, along with mild neurocognitive and ophthalmologic events. It was also observed that a higher proportion of patients had low vitamin E or K levels, although these were not clinically significant. The primary limitation to their broader use is their high cost (29).

PSCK-9 inhibition by siRNA-based therapeutic agents: inclisiran

In recent years, RNA-based therapies have undergone significant development. This therapy involves the management or prevention of diseases using RNA-based molecules. RNA molecules are classified into coding RNAs and non-coding RNAs (ncRNAs). NcRNAs, which do not encode functional proteins, include various types, such as small interfering RNAs (siRNAs). Small interfering RNA molecules exert possibility as treatment options for a variety of diseases, including cancer, viral and bacterial infections (52). They have demonstrated successful inhibitory effects on tumor growth, along with high specificity, low adverse effects, safety, and high efficiency even at very low doses. SiRNAs are resistant to nuclease enzymes degradation in contrast to antisense agents (53). The safety of siRNAs refers to the absence of harmful chemicals in the siRNA synthesis processes (54). Unlike other antisense agents, siRNA exerts its inhibitory effects on gene expressions during the post-translation stages without interfering with DNA or inducing any mutation in its structure (55).

The first treatment based on siRNA, received approval by the Food and Drug Administration (FDA) in 2018. In 2020 Leqvio®, (inclisiran 284 mg/1.5 mL) was put on the map by the European Medicines Agency and by the United States Food and Drug Administration in 2021 for primary hypercholesterolemia or mixed dyslipidemia (52,53). As mentioned above, Inclisiran is a small interfering ribonucleic acid (siRNA) that inhibits the synthesis of PCSK-9 in hepatocytes. It is approved for the treatment of patients with HeFH, familial combined hyperlipidemia and confirmed atherosclerotic CVD (13,30,56). Inclisirans mechanism of action is linked to the activation of the RNA-induced silencing complex (RISC), which cleaves intracellular mRNA, thus blocking translation and preventing the synthesis of PCSK-9. The result of these actions is the reduction in circulating levels of PCSK-9 by up to 70% (37). SiRNA consists of two complementary RNA strands, an antisense (guide) strand, and a sense (passenger) strand. The siRNA enters the hepatocytes with precise and rapid hepatic uptake (53). When the siRNA is loaded into the RNA induced-silencing complex (RISC) in hepatocytes, only the antisense strand becomes activated, as the sense strand is selectively removed by the Argonaut 2 (57). The complex formed by the antisense strand and RISC binds to PCSK9 messenger RNA (mRNA) transcripts, selectively and catalytically cleaving the mRNA. This process inhibits the translation of complementary mRNA transcripts. Every complex exabits an extended half-life, enabling it to degrade multiple mRNA copies, which allows for dosing intervals of several months in patients. It is quickly cleared from the circulation via hepatic uptake and renal excretion, becoming undetectable in the plasma by 48 h post-delivery (53). With PCSK-9 inhibition, there is an "up regulation" of LDLR resulting in the clearance of LDL-C (13,37,44). It is delivered via subcutaneous injection at a dose of 284 mg. Following the initial dose, the second dose is given after three months, with following doses administered every 6 months (44,56). In comparison to PCSK-9 monoclonal antibodies, inclisiran has a more convenient dosing regimen, it is given twice a year, guaranteeing better compliance (13,56). The ORION studies examined the efficacy and safety of the drug in patients receiving highest tolerated doses of statins and other lipid-lowering drugs. Inclisiran has been shown to reduce LDL-C by about 50% compared to the placebo group following a one-year follow-up (13,56,58). Like monoclonal antibodies, inclisiran also promotes lowering of total cholesterol, Tg, non-HDL-C, Lp(a) and as well increasing HDL-C (58). The ORION-4 and VICTORION-2 PREVENT studies are large long-term studies that will assess the incidence of new CVD in patients with confirmed atherosclerotic CVD who were previously on statin therapy, and these studies are still ongoing. The main endpoint of these studies is expected to provide results on the reduction of major cardiovascular advance (MACE) in patients on inclisiran therapy (56,58).

Data from three pivotal Phase III placebo-controlled ORION trials not only assessed the lipid-lowering efficacy of inclisiran, but also evaluated its impact on major adverse cardiovascular events (MACE). Each trial enrolled patients at high risk of CV events who, although receiving the highest tolerated doses of statins, continued to have elevated LDL-C levels. The study population consisted of patients with HeFH (ORION-9), ASCVD (ORION-10, ORION-11), and high-risk, primary prevention patients referred to as ASCVD risk equivalent (ORION-11). Patients in the ORION-11 trial included individuals with no prior history of ASCVD, but who had either type 2 diabetes mellitus or HeFH, or a predicted 10-year CVD risk of >20% based on the Framingham risk score or equivalent (59). The data demonstrated that adding inclisiran to existing lipid-lowering therapies was associated with a 26% reduction of MACE. Favorable trends towards a reduced risk of both fatal and non-fatal MI in comparison to placebo were also observed. Strokes were numerically less present in the inclisiran treatment arm.

The exposure tame in these studies was 18 months, but to fully assess the benefit of lipid-lowering therapy with PCSK-9 inhibitors on CV events, we need greater long-term absolute reduction of LDL-C levels (59).

The ORION-5 trial was a double-blind, placebo-controlled, open-label, multicenter trial, assessing the long-term effects, tolerability, and safety of inclisiran in HoFH patients. Results showed that inclisiran did not significantly reduce LDL-C and was not superior to placebo in patients already on maximal lipid-lowering medications. HoFH patients with higher residual LDLR function may experience greater LDL-c reduction in comparison to patients with lesser residual LDLR function (60). The ORION-13 and -16 trials, designed to evaluate the efficacy and safety of inclisiran in adolescents aged 12 to 17 years with HoFH and HeFH, are currently in progress (61).

It has been speculated that inhibition of hepatic PCSK-9 synthesis may have greater benefits in CVD prevention compared to monoclonal antibodies alone, especially in the light of their well-known pleiotropic effects (37). However, there is not enough evidence to substantiate these findings. We are waiting for future studies to provide the answers.

Regarding the safety profile of inclisiran, pooled data from 7 clinical trials represent the largest data set to date, demonstrating that twice-annually injections are both safe and well tolerated when used in conjunction with statins and/or other oral lipid lowering agents in patients with increased levels of LDL-C, including those with ASCVD, ASCVD risk equivalent, and HeFH (62). The majority of reported adverse events were mild and moderate, including myalgia, cough, mild rash, hyperpigmentation, headache, nasopharyngitis, and dizziness (53). In the ORION 3, with four years of extended exposure, the most frequently occurring adverse events in the inclisaran arm were nasopharyngitis reported in 19% of patients, and injection site reactions in 14% of patients (63). In contrast to monoclonal antibody that resides in blood, siRNA is quickly absorbed by the liver. Repeated exposure to inclisiran leads to sustained reduction of circulating PCSK9 levels, with impacts spanning from 62,2% to 77,8% over 4 years, without any indication of compensatory mechanisms or escape phenomena that would compromise its LDL cholesterol lowering efficacy (63). Possible harmful metabolic effects of inclisiran, as suggested by latest meta-analysis of genetic association studies, indicated to increased probability of developing type 2 diabetes (64).

NOVEL AND FUTURE LIPID-MODULATING THERAPY BASED ON PCSK-9

Oral PCSK-9 inhibitor: MK-0616

MK-0616 is an oral PCSK-9 inhibitor, at present in a phase 3 of randomized, double-blind, placebo-controlled study. The aim of the study is to assess the efficacy and safety of this macrocyclic peptide after 24 weeks in adults with hypercholesterolemia. First results showed that orally administered PCSK-9 inhibitor in a dose of 6 to 30 mg resulted in a decrease of LDL-C up to 60.9% in comparison to placebo following designated follow-up period of 8 weeks in patients with hypercholesterolemia and ASCVD on statin therapy (65). CORALreef Outcomes (TIMI 77) is a randomized, double-blind study whose aim is to evaluate the efficacy of MK-01616 in patients with high CV risk on statin therapy versus a placebo group. Preliminary results of this study will come out on November 2029, and it will aim to answer weather therapy with MK-0161 will prolong the time of onset of new CV event (66).

PCSK-9 vaccines

PCSK-9 vaccines are liposomal immunogenic combination of PCSK9-tetanus peptide with an aluminum adjuvant, which in an animal model showed long-term synthesis of PCSK-9 antibodies and reduction of LDL-C and VLDL-C by 51.7% in BALB/c mice and 19.2% in C57BL/650 mice (67).

Gene therapy

CRISPR/Cas system (clustered regularly interspaced short palindromic repeats) is a technology that changes the function of the PCSK-9 gene making it non-functional (68). VERVE-101 is a mRNA packed in lipid nanoparticles silencing the gene for PCSK-9. This innovative molecule is being evaluated in phase 1b of Heart-1 clinical trial in patients with high-risk HeFH, established ASCVD and uncontrolled LDL-C levels despite patents being on maximum doses of oral therapy. Early-stage research conducted in mice and non-human primates (NHP) have demonstrated that a single intravenous administration of VERVE-101 can effectively inactivate the PCSK-9 gene in the liver (69). The first results of Heart-1 study were presented at the AHA Scientific Sessions in November 2023 and they showed that the NHPs treated with VERVE-101 achieved a marked decrease in LDL-C up to 55% and lower blood PCSK-9 protein up to 84% that remained permanently reduced during more than two years of follow-up. VERVE-101 is believed to be a single-course therapy capable of producing profound LDL-C reduction lasting for decades. The initial clinical trial conducted in volunteers with HeFH and developed ASCVD is ongoing (69).

CONCLUSION

The positive role of PCSK-9 inhibition in dyslipidemia therapy and ASCVD prevention is clearly shown in several randomized clinical studies. The mechanisms of inhibition of the PCSK-9 molecule will probably be further refined in more depth in the future, and time will allow

the development of additional therapeutic possibilities. For now, indications for PCSK-9 use are clear, but they will be expanded in further investigations. Due to the exceptional effectiveness of this group of drugs, it is believed that the results of ongoing studies will answer even more precisely their importance and the possibility of application in patients immediately after a cardiovascular event during hospital treatment, as well as their benefits or potential risks in patients with renal or hepatic insufficiency.

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Ethical approval: N.A.

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TERAPIJA DISLIPIDEMIJE: PCSK-9 U FOKUSU

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Sažetak

Kardiovaskularne bolesti su vodeći uzrok morbiditeta u svetu i čine trećinu svih uzroka smrtnosti. Dislipidemija kao najznačajniji faktor rizika ima značajnu ulogu u nastanku i progresiji aterosklerotske kardiovaskularne bolesti. Terapija statinima često ne uspeva da obezbedi ciljne vrednosti LDL holesterola, a i kada se one postignu, kardiovaskularni događaji se i dalje javljaju, što ukazuje na potrebu za razvojem i primenom novih lekova za snižavanje lipida. PCSK-9 inhibitori redukuju LDL holesterol za oko 60% kada se koriste kao monoterapija i čak 85% u kombinaciji sa statinima visokog intenziteta i/ili drugim lekovima za lečenje dislipidemije. Alirokumab i evolokumab su monoklonska antitela koja se efikasno vezuju za LDL receptor ometajući njegovu razgradnju. Inklisiran je mala interferirajuća RNK koja ometa sintezu PCSK-9 molekula. Indikaciona područja za primenu PCSK-9 inhibitora se sve više šire zbog otkrića njihovih plejotropnih efekata. Takođe, u fokusu je otkriće novih mehanizama PCSK-9 inhibicije što bi otvorilo vrata razvoju novih terapijskih agenasa. Studije efikasnosti i bezbednosti oralnih PCSK-9 inhibitora su u toku, kao i rane faze kliničkih ispivanja genske terapije.

Cilj ovog preglednog članka je da predstavi dosadašnja saznanja o primeni PCSK-9 inhibitora.

Ključne reči: dislipidemija, LDL holesterol, PCSK-9

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REVIEW ARTICLE

Importance of physical activity in people with multiple sclerosis

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Summary

Physical activity (PA) in people with multiple sclerosis (pwMS) is related to positive effects on numerous bodily functions such as mobility, balance, fatigue, cognition and improves their overall quality of life. Recent research pointed out possible effects of PA on disease progression and even on the risk of developing the disease. Despite these facts, pwMS are still less active than general sedentary population, so improving their participation in PA should be one of the very important goals in their treatment.

In order to adequately tailor PA goals, pwMS should be referred to rehabilitation specialist from the initial stage of the disease. It appears that programs that include walking are much appreciated by pwMS, so perhaps they should be prioritized in all patients with mild and moderate disability levels. Special attention is needed for the group of patients with severe disability in order to improve availability of venues and trained assistants that can help them stay maximally active.

Barriers to reduced PA stem from the patient's health status, cognitive and behavioral factors on one hand, and the physical and social environment on the other. For that reason, it is very important to include patient's preference for a modality of PA when proposing PA plans. Long-term adherence to PA programs remains insufficiently studied, so further research is needed to address this important issue, which appears to be a key step toward improving PA engagement in pwMS.

Keywords: physical activity, multiple sclerosis, exercises.

INTRODUCTION

Physical activity (PA) is related to multiple health benefits, physical and psychological, demonstrated in the general population as well as in patients with different chronic diseases (1). General health benefits include lower risk for cardiovascular diseases, diabetes mellitus, obesity, cognitive dysfunction, and in the elderly population, PA is related to diminished risk of falls and frailty (2-4).

Physical activity is defined as bodily movement produced by the contraction of skeletal muscles, which causes substantial energy expenditure compared to the resting state (5). It includes activities related to work, household, leisure time, transport, sports activities, and exercise. Exercises are a form of PA that is planned, structured, and repetitive, with a goal of improving or maintaining physical fitness (5). So far, there is no clear evidence on the different effects of exercises compared to PA, and they are generally considered to have comparable effects if performed at moderate to high intensity, as has been shown for outcomes of physical function in older healthy individuals (6).

People with multiple sclerosis (pwMS) are less physically active than the sedentary general population (7), which is to be expected, considering their difficulties in walking, balance, and fatigue. Insufficient PA additionally worsens their physical condition by causing muscle weakness and deconditioning, but also leads to more pronounced neurological symptoms and signs of disease, which could be influenced by engaging in PA. Furthermore, in order to obtain and preserve the health benefits of PA and exercise, regular activity throughout one's life is necessary. For this reason, much of the research in recent years has been oriented towards a better understanding of the preferences of pwMS for PA, barriers, and strategies for improving it (8, 9).

Our research aimed to synthesize existing knowledge on this topic, both the benefits and barriers, in order to offer a comprehensive review for healthcare practitioners.

METHODS

A search of PubMed and PEDro databases was performed in order to identify studies related to the effects of PA in multiple sclerosis, including recommendations, facilitators, and barriers to PA, published in the English language within the last 15 years. This encompassed experimental and clinical trials, as well as reviews on the topic.

EFFECTS OF PHYSICAL ACTIVITY AND EXERCISE

The effect of exercises on symptoms and signs of disease (tertiary prevention) has been well known for years and is a mainstay of rehabilitation programs. There is a large body of evidence on the positive effects of different types of exercises on mobility, balance, muscle strength, endurance, fatigue, and quality of life (10, 11). Data from available systematic reviews and meta-analyses suggest that general exercises can positively influence most of the bodily functions listed by pwMS as most important (fatigue, pain, walking, balance, cognition) (11). When analyzing separate effects of different exercise modalities, there is a positive effect of resistance and aerobic training on most of the bodily functions (12), while evidence for yoga and Pilates is still scarce (13, 14).

Furthermore, the impact of exercise and PA might extend beyond influencing symptoms and signs of disease. There is emerging evidence for its potential secondary and even primary prevention effect (11). Disease-modifying effect of exercise and PA (secondary prevention) is mainly shown in studies on animal models (15-17). In pwMS, few interventional studies demonstrated brain MRI changes related to exercise, such as improved functional connectivity, viscoelasticity, and white matter plasticity (18-21). Some clinical studies have shown a decreased relapse rate, but this effect was inconsistently reported, as it was not the primary outcome in any of the studies (22).

The relationship of PA and decreased risk of developing MS (primary prevention) is shown in two extensive case-control studies, where moderate to high intensity physical activity was related to a lower risk of developing multiple sclerosis (23, 24). Although the causality can be questioned, these results are valuable, as they highlight a strong association that warrants further analysis.

Regarding explanatory mechanisms of exercise-induced symptomatic, disease-modifying, and preventive effects, existing data are based on its neuroprotective role. The majority of research in this field has demonstrated a relationship between exercise-induced changes in reducing inflammation and/or increasing the expression of neurotrophic factors (brain-derived neurotrophic factor (BDNF), in particular) and the preservation of CNS structure and function (15, 16, 25). It is also believed that the effect of exercises could be through preservation of the brain blood barrier and cerebral perfusion; however, more data on this potential mechanism are still expected from future studies (26, 27).

PHYSICAL ACTIVITY RECOMMENDATIONS

Current recommendations for PA in pwMS suggest a minimum of 150 minutes of exercise per week and/or 150 minutes of lifestyle PA (28). Lifestyle PA includes at least 30 minutes of moderate intensity PA that is accumulated through different daily activities (29). Exercise or PA recommendations in this guideline (28) are stratified according to the Expended Disability Status Scale (EDSS), and the type and intensity of exercises vary depending

on the spectrum of neurological signs and symptoms. An early evaluation of patients is suggested for tailoring an individualized activity plan. The organization of physiotherapy in Europe differs a lot (30), so patients should first be evaluated by an available MS specialist (rehabilitation physician, physiotherapist, occupational therapist, or sport scientist, all experienced in this field), depending on the healthcare system organization. For people with lower disability levels, whose mobility and balance are not impaired, any kind of activity can be performed, with an emphasis on undertaking moderate to high-intensity activity within a sufficient time period. With the increase in disability, a specialist referral is necessary to obtain an appropriate exercise or activity plan prescription. When mobility is limited, exercise should be performed with the assistance of a trained professional. Progression towards the aimed PA levels should be gradual and in accordance with the patient's preferences, capabilities, and safety considerations (28).

PHYSICAL ACTIVITY FROM THE PATIENTS' PERSPECTIVE

Despite the acknowledged benefits of PA, there is still a substantial gap in its adoption by pwMS. Research directed towards patients' perspectives is therefore a valuable resource for the analysis of reasons related to such discrepancies. The study that showed consensus made PA preferences in community-dwelling pwMS (9) demonstrated that highly prioritized activities were self-care, daily life, and domestic activities, while stretches, physiotherapy, and non-weight bearing exercises were at the bottom of top ten activity list. Activities such as strengthening exercises, endurance training, yoga, and Pilates were eliminated during the first rounds of consensus. Looking for the reasons for engagement in PA, in the same study, the consensus ranking showed improving MS symptoms as the most important reason, followed by categories such as "out of necessity"," to keep active", and "to keep walking". Although including a relatively small number of participants, the data from this study offered, for the first time, consensus-based answers generated in the community-dwelling population of pwMS, underlining differences between recommendations given by experts and preferences of pwMS.

A large body of evidence on PA in pwMS was collected during the COVID pandemic from an extensive international survey study that included 11 countries and more than 3000 respondents (31-33).

During a pandemic, there was a concern that already reduced PA would further diminish due to restrictions and fear of contracting the infection, so much effort was put into research in order to minimize this potential negative impact. Although this study aimed to register PA during the COVID pandemic, it also gave us important data on pre-pandemic PA of pwMS. The majority of respondents (83%) reported being physically active pre-pandemic, mainly participating in PA of moderate intensity (31). The most practiced activity was walking for mild and moderate levels of disability, followed by exercises in the gym and physiotherapy as a dominant way of activity for severely disabled persons (31). As for the use of technology, the majority (65%) did not use any of it, and among those who did, only the use of wearable devices such as pedometers or smart watches was notable (31). Existing recommendations for minimum PA were fulfilled by 64% of patients with mild disability level, 51% of moderate disability, and only 39% of severely disabled (32). Special concern emerged regarding low percentage of patients willing to change their physical activity habits among the physically inactive (31).

BARRIERS AND FACILITATORS OF PHYSICAL ACTIVITY

Although research data showed high adherence to exercises in a short-term training period (12), data on adherence in a long-term period are scarce. As pwMS are insufficiently physically active, adherence to exercise is one of the main questions to address, in order to improve PA. Rehabilitation, PA, and exercises are behavioral interventions that include educational and interactive treatment in order to promote healthy behavior change. Very little data are available on approaches for initiating and then sustaining change in PA, proposing different models such as: group training, socializing via internet exchanges, application of gaming settings, health education, financial motivation, but with no apparent efficacy of any of them (34). Some research pointed out the importance of patient–clinician interaction and knowledge translation into clinical practice by health care providers, as one of the most essential ways for the promotion and maintenance of PA in pwMS (12). It is shown that pwMS are interested in getting information about exercising, searching for materials supporting home and community-based exercising and seeking for interaction with health care providers, particularly trough face to face interaction, in initiating and maintaining PA (35). On the other hand, health care providers identified having opportunities for exercise promotions and were looking for educational resources, protocols and guidelines for promotion of behavior change in people with multiple sclerosis (36). Therefore, there is an emerging need to put effort in better organization of health care system, to enable dissemination of information in order to enhance PA of pwMS.

Barriers to PA are numerous and should be carefully assessed when making PA plans. They are put in six categories and include: physical and social environment, health condition, cognitive or behavioral components, costs, and time (28, 37). Physical environment includes

rural versus urban environments, home environment, community facilities, parking access, transportation, and temperature (climate) (28). Social environment is related to limited support from providers or family, exclusion, dependence, social stress, attitudes of others, cultural factors, and socioeconomic factors (28). Cognitive /behavioral factors include fear, poor self-management, frustration, low confidence, depression, impaired memory, planning, prioritizing, and focus (28). Research conducted during the COVID-19 pandemic identified several factors that contributed to increased physical activity (PA), including public messaging on the importance of staying active, increased free time, greater family support, improved daily structure, and reduced costs (31). These findings reinforce the previously identified barriers to PA.

CONCLUSION

Based on existing data, it is obvious that there is still much to be done in order to improve PA level among pwMS. Staying active is one of the most important messages that should be presented to all patients, from the initial contact with the specialist, with all the benefits clearly explained. In order to help patients, preserve a physically active lifestyle, the importance of specialists and institutional support, and collaboration between them is crucial. It seems that programs that include walking are much appreciated by pwMS so maybe they should be prioritized in all patients with mild and moderate disability levels. Special attention is needed for the group of patients with severe disability in order to improve their physical activity level. This includes better availability of outpatient physiotherapy centers, as well as local community and patients'organization engagement in ensuring venues, equipment, and trained assistants. Patients' preferences regarding the modality of PA have to be taken into account, as there are still many discrepancies between experts' recommendations and patients' perspectives. Therefore, much work lies ahead, especially to warrant long-term adherence to proposed physical activity plans, as it seems to be one of the biggest challenges for improving PA in pwMS.

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VAŽNOST FIZIČKE AKTIVNOSTI KOD OSOBA OBOLELIH OD MULTIPLE SKLEROZE

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Sažetak

Fizička aktivnost (FA) osoba sa multiplom sklerozom (oMS) je povezana sa brojnim pozitivnim efektima na telesne funkcije kao što su mobilnost, balans, zamor, kognicija i poboljšava sveukupni kvalitet života obolelih. Skorašnja istraživanja ističu i mogućnost uticaja FA na progresiju bolesti, pa čak i na smanjenje rizika od njenog nastanka. Uprkos ovim činjenicama, oMS su i dalje manje fizički aktivne od opšte populacije sa sedentarnim načinom života., te poboljšanje FA treba da bude jedan od važnih ciljeva u sklopu njihovog lečenja.

U cilju adekvatnog kreiranja ciljeva vezanih za poboljšanje FA, oMS treba uputiti specijalisti rehabilitacione medicine od samog početka bolesti. Programi koji uključuju hodanje, jedan su od vidova FA koji oMS preferiraju i trebalo bi ih prioritizovati kod obolelih sa lakim i srednjim stepenom onesposobljenosti. Posebnu pažnju takođe treba usmeriti na obolele sa teškim stepenom onesposobljenosti u smislu poboljšanja dostupnosti prostora i pomoći, što bi omogućilo veći stepen fizičke aktivnosti ove grupe pacijenata.

Prepreke vezane za smanjenu FA potiču sa jedne strane od zdravstvenog stanja obolelih, njihovih kognitivnih i bihejvioralnih karakteristika, a sa druge strane, vezani su za uslove sredine i socijalni kontekst. Zbog svega toga, pri planiranju fizičkih aktivnosti veoma je važno uključiti obolele i uzeti u obzir njihove afinitete ka određenom modalitetu FA. Adherenca obolelih za programe FA u dužem vremenskom periodu nije dovoljno ispitana. Ovaj faktor smatra se jednim od najvažnijih za povećanje FA i fokus budućih istraživanja treba usmeriti ka njegovom ispitivanju.

Ključne reči : fizička aktivnost, multipla skleroza, vežbe

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CASE REPORT

Successful use of delayed therapeutic plasma exchange in seronegative pediatric autoimmune encephalitis

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Summary

Introduction: We present a 5-year-old girl with severe seronegative autoimmune encephalitis (AE) who was treated with therapeutic plasma exchange (TPE) after unsuccessful treatment with corticosteroids and intravenous immunoglobulins (IVIG).

Case report: We performed 6 TPEs every other day in the Intensive Care Unit (ICU) using a Spectra Optia apheresis system and a central venous catheter for vascular access. We used a 5% albumin for substitution and acid-citrate dextrose-A (ACD-A) solution for anticoagulation. The exchange set was primed with allogenic compatible red blood cells because the patient weighed < 20kg. Before each TPE, we checked the patient's blood count and coagulation status. A slow intravenous infusion of calcium gluconate (30mg/kg/h) was administered to maintain ionized calcium levels above 1 mmol/L. The mean inlet flow rate was 22.5 ± 4.1 ml/min, and the processed total blood volume was 2794.5 ± 106.0 ml. On average, 1243.2 ± 49.2 ml (1.55 ± 0.05) total plasma volume was exchanged, with 288.8 ± 13.8 ml of ACD used. The mean procedure duration time was 137.2 ± 9.2 min. After the third procedure, significant clinical improvement was observed in the girl. She smiled purposefully, followed with her gaze, and focused on the audiovisual content adapted for her age. Two weeks after the last TPE, the girl was discharged in good clinical condition from the hospital. Three years after the treatment, the patient was in good general condition with normal neurological status and without sequelae.

Conclusion: This case highlights the therapeutic effectiveness of TPE, even when initiated late, in pediatric patients with severe AE.

Keywords: therapeutic plasma exchange, autoimmune encephalitis, pediatrics

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INTRODUCTION

Autoimmune encephalitides (AE) are disorders characterized by various clinical manifestations – seizures, psychiatric and behavioral symptoms, movement disorders, and autonomic disturbances. It results from the immune response and the creation of antibodies to neuron antigens. According to the latest literature data, the incidence of AE is 1.54 children/million with a female predominance (1-3). Some forms of AE are more prevalent in children and young adults, like anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis and acute disseminated encephalomyelitis (ADEM)(4). The presence of antibodies against neuronal cell-surface proteins, receptors, and ion channels characterizes these forms of AE. The clinical presentation of AE can be very diverse. One of the most common symptoms is various types of epileptic seizures, including epileptic status. In the subacute phase of the disease, which can last from a few days to a few weeks, fluctuations in the state of consciousness are noticeable (from a gradual decrease in the level of consciousness up to coma) and cognitive functions. Abnormal movements such as ataxia, dystonia, and myoclonus can also be one of the leading symptoms. Based on the severity of symptoms, first-line therapies include intravenous steroids, intravenous immunoglobulins (IVIG), and/or therapeutic plasma exchange (TPE). Rituximab or cyclophosphamide is the second-line treatment in refractory cases (1). TPE's role in treating AE is well-established and recommended. For NMDAR encephalitis, TPE is the first option (5). The efficacy of TPE relates to the reduction of titers in circulating autoantibodies and the consequent immunomodulatory effect (6).

This work aimed to show that delayed treatment with TPE could also be effective in a severe seronegative form of AE in a 5-year-old child.

The publication of this case report was reviewed and approved by the Ethics Committee of the Mother and Child Health Care Institute of Serbia "Dr.Vukan Čupić", Belgrade, approval number 8/87/25 (date July 22, 2025). The patient's parents gave written informed consent to publish the details of the patient's medical case and any accompanying images.

CASE REPORT

A female patient, a 5-year-old, was admitted to the Institute with severe encephalopathy preceded by fever. The illness began two weeks before her admission, characterized by an elevated body temperature of up to 38.3°C and general weakness. The girl was admitted to the regional health center for rehydration. A few hours after her admission, she experienced an episode of agitation when she bent forward from a supine to a sitting position with clenched fists. A similar episode occurred a few hours later. Following this, the girl exhibited blurred consciousness, a staggered gait, and instability, without any verbal communication. During this episode, her gaze was fixed to the left. Intravenous hydration was initiated, along with antiedematous treatment (20% mannitol at 0.25 mg/kg and dexamethasone at 0.4 mg/kg), and antimicrobial therapy (Ceftriaxone and Acyclovir). Due to the persistence of neurological deficits and only slight clinical improvement, the girl was transferred to the Institute for further care.

The mother recalls that her daughter received all vaccinations on schedule and developed normally until six months old, when she began experiencing episodes of cyanosis and a fixed gaze. Doctors recommended rectal diazepam for her febrile episodes. At eight months, during another fever, she had tonic-clonic convulsions. Initially treated with phenobarbital, she was switched to valproic acid (VPA) due to side effects. Remarkably, she remained seizure-free for three years on VPA. Although the medication was stopped six months before her current condition, she stayed seizure-free during that time.

After admission to our Institute, the patient's clinical condition indicated encephalitis, and appropriate empiric therapy was applied. We treated her initially with triple antimicrobial therapy (ceftriaxone, erythromycin, acyclovir) for 21 days, antiedematous (mannitol), and anti-inflammatory (dexamethasone) therapy for six days. Severe coma persisted despite normal cytological and biochemical findings in the cerebrospinal fluid (CF), and therefore, an immune-mediated etiology of encephalitis was suspected. On the second day of hospitalization, we started with a five-day methylprednisolone pulse therapy, followed by oral prednisone 2mg/kg. The microbiological (Herpes simplex virus, Coxsackievirus B1-B5, West Nile Virus, Mycoplasma pneumoniae, Borrelia burgdorferi) and immunological (IgA, IgM, IgG, C3, C4, antiphospholipid, anticardiolipin, ANA, pANCA, cANCA, anti-dsDNA antibodies) analyses of serum and CF were negative. The specific antibodies for AE and anti-NMDAR were also negative. An additional metabolic examination was performed, which included screening of urine, plasma amino acids, and organic acids in the urine. All test results were normal. The endocranium MRI was normal. In the second week of hospitalization, due to a lack of clinical improvement, we started with intravenous immunoglobulin (IVIG) therapy (2mg/kg/48h). Despite the applied therapy, the girl's condition continued to deteriorate. Generalized hypotonia of the musculature and right-sided hemiparesis with greater hand involvement were observed. There were also many involuntary movements. Therapy with haloperidol (1mg/daily) and lorazepam (1mg/daily) was started, which gradually led to improvement in behavior and reduction of involuntary movements. After four weeks of hospitalization, the patient received another pulse dose of methylprednisolone (500mg/m2). Despite the applied immunomodulatory

and immunosuppressive therapy, there was no clinical improvement, so the team of physicians decided to start the treatment with TPEs.

We started with TPE treatments in the ninth week of hospitalization, which was seven weeks after IVIG. The patient weighed 16 kg and was 110 cm tall, with 1280 ml of total blood volume, 742.4 ml of plasma volume, and hematocrit (Hct) 0.42 L/L. We performed 6 TPEs every other day in the ICU using a Spectra Optia apheresis system with continuous centrifugation and a central venous catheter for vascular access. We used a 5% albumin solution for substitution and acid-citrate dextrose-A (ACD-A) solution for anticoagulation. The exchange set was primed with allogenic compatible red blood cells because the patient weighed less than 20 kg. Before each TPE, we checked the patient's blood count and coagulation status. A slow intravenous infusion of calcium gluconate (30mg/kg/h) was administered to maintain ionized calcium levels above 1 mmol/L. **Table 1** shows the characteristics of the procedures performed.

Table 1. Therapeutic plasma exchange (TPE) procedures characteristics

Procedure characteristics	Mean ± SD
IF rate (ml/min)	22.5 ± 4.1
TBV processed (ml)	2794.5 ± 106.0
PV exchanged (ml)	1243.2 ± 49.2
Total PV exchanged (L)	1.56 ± 0.05
Acid citrate dextrose used (ml)	288.8 ± 13.8
Procedure duration time (min)	137.2 ± 9.2
Ionized calcium before TPE (mmol/L)	0.90 ± 0.22
Ionized calcium after TPE (mmol/L)	1.0 ± 0.08
Hematocrit value before TPE (L/L)	0.38 ± 0.02
Hematocrit value after TPE (L/L)	0.41 ± 0.02

IF – inlet flow; TBV – total blood volume; PV – plasma volume; Mean – arithmetic mean; SD – standard deviation; TPE - therapeutic plasma exchange

There were no side effects during the procedures. After the third procedure, significant clinical improvement was observed in the girl. She smiled purposefully, followed with her gaze, and focused on the audiovisual content adapted for her age. After conducting six TPE procedures, the immunosuppressive drug azathioprine was introduced into the treatment plan. In addition to the drug therapy mentioned earlier, daily physical therapy and cognitive stimulation were conducted in collaboration with the girl's mother. Two weeks after the last TPE, the girl was discharged in good clinical condition from the hospital. She underwent physical therapy, resulting in significant clinical improvement. She communicated using complex sentences, and her motor skills were significantly better. During the most recent check-up, three years after undergoing treatment at our Institute, the patient was in good general condition with normal neurological status and without sequelae.

DISCUSSION

Various pediatric neurological disorders can be successfully treated with TPE. It primarily refers to pediatric immune-mediated diseases such as autoimmune encephalitis, acute and chronic polyneuropathy, acquired demyelinating diseases, paraneoplastic syndromes, and inflammatory vascular diseases of the CNS (7,8). With the advancement of technology, apheresis systems have become suitable for children with low body weight. Apheresis devices used nowadays (Spectra Optia, Amicus) have a smaller extracorporeal volume than older machines. The understanding of the TPE mechanism and the knowledge of the indications for its use by clinicians are also increasing. Thanks to these facts, we have expanded this immunomodulatory therapeutic approach to treat many pediatric neurological disorders.

The literature lacks consistent data on the use of TPE in treating pediatric AE, making it challenging to assess TPE's advantages compared to other immunomodulatory therapies.

Various responses to TPE in treating AE in children were demonstrated in previous studies. In their research, Atay et al. reported mild improvement in 5 out of 20 patients diagnosed with AE (7). The cross-sectional survey of Nikkhah et al. examined a cohort of 18 children with neuroimmunological diseases who underwent TPEs. The AE group, consisting of four patients, exhibited the most favorable outcomes in this study (9). In their research, Günay Ç et al. included ten pediatric patients with acute neurological conditions treated with TPEs. Two patients in this study demonstrated complete recovery after TPEs, and one had AE (10).

Our patient showed significant neurological improvement after three TPEs, even though they were applied late, in the ninth week of hospitalization. That leads us to think that some undetermined antibodies were present in her plasma, but we could not determine their specificity at the time. The list of potential antibodies involved in the pathogenesis of AE is constantly expanding and may still need to be completed. In addition, TPEs remove various other disease mediators from plasma, contributing to successful treatment with immunomodulatory effects on the CNS. Although the temporal association with TPE is clear, an immunomodulatory cumulative impact of prior corticosteroid and IVIG therapy cannot be excluded.

Our study needed to be improved in this regard because we didn't examine the presence of other antibodies that could be associated with AE. After all, they were not available to us at that time.

CONCLUSION

Even late treatment with TPEs can contribute to promising outcomes in treating pediatric patients with AE. TPE

acts as an immunomodulatory intervention, modifying immune components and removing antibodies. No adverse events related to TPE were observed. It is a safe and very effective procedure for autoimmune neurological disorders in children.

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Data Availability: Data could be obtained from the corresponding author upon request.

Ethical approval: The publication of this case report was reviewed and approved by the Ethics Committee of the Mother and Child Health Care Institute of Serbia "Dr.Vukan Čupić", Belgrade, approval number 8/87/25 (date July 22, 2025). The patient's parents gave written informed consent to publish the details of the patient's medical case and any accompanying images.

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USPEŠNA PRIMENA ODLOŽENE TERAPIJSKE IZMENE PLAZME KOD PEDIJATRIJSKOG SERONEGATIVNOG AUTOIMUNOG ENCEFALITISA

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Sažetak

Prikazujemo petogodišnju devojčicu sa teškim seronegativnim autoimunim encefalitisom koja je lečene terapijskim izmenama plazme (TIP), nakon neuspešne primene kortikosteroida i intravenskih imunoglobulina (IVIG). Sproveli smo ukupno 6 TIP svakog drugog dana u odeljenju intenzivne nege na Spectra Optia afereznom sistemu uz korišćenje centralnog venskog katetera za vaskularni pristup. Koristili smo 5% rastvor albumina za supstituciju i Acid Citrate Dextrose – A (ACD-A) rastvor za antikoagulaciju. Set za izmenu plazme je ispunjavan alogenim kompatibilnim eritrocitima jer je devojčica imala < 20kg. Pre svake TIP, kontrolisali smo krvnu slika i koagulacioni status. Vrednosti jonizovanog kalcijuma su održavani iznad 1mmol/L primenom spore intravenske infuzije kalcijum glukonata (30mg/kg/h). Srednja vrednost brzine utoka bila je 22.5 ± 4.1 ml/min, procesiranog ukupnog volumena krvi 2794.5 ± 106.0 ml. U proseku je izmenjeno 1243.2 ± 49.2 ml (1.55 ± 0.05) volumena plazme i upotrebljeno 288.8 ± 13.8 ml ACD-A. Procedure su u proseku trajale 137.2 ± 9.2 min. Nakon treće procedure, zapaženo je značajno kliničko poboljšanje. Devojčica se svrsishodno osmehivala, pratila pogledom i fokusirala na audiovizuelne sadržaje prilagođene njenom uzrastu. Otpuštena je iz bolnice dobrog opšteg stanja, dve nedelje nakon poslednje TIP. Na poslednjoj kontroli, tri godine nakon lečenja, devojčica je bila dobrog opšteg stanja, sa normalnim neurološkim statusom, bez sekvela. Ovaj prikaz slučaja ističe terapijsku efikasnost TIP kod pedijatrijskih pacijenata sa teškim AE čak i kada se započne kasnije.

Ključne reči: terapijska izmena plazme, autoimuni encephalitis, pedijatrija

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