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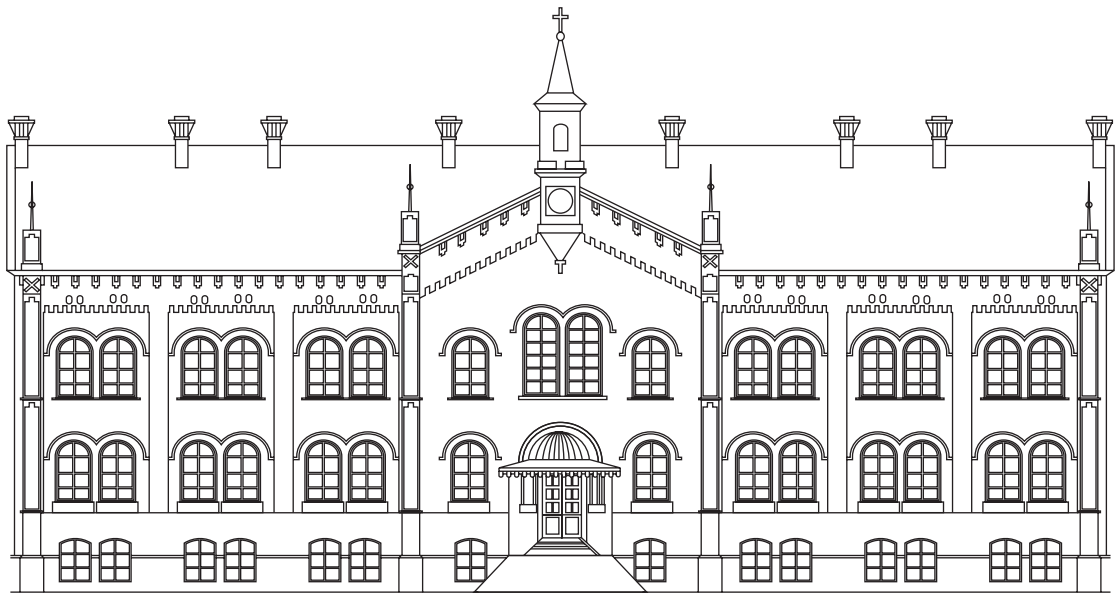

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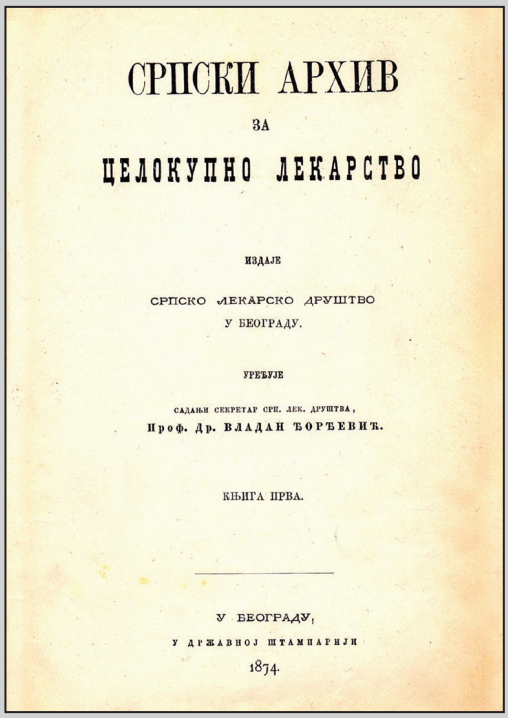

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

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САДАШЊИ СЕКРЕТАР СРП. ЛЕК. ДРУШТВА,

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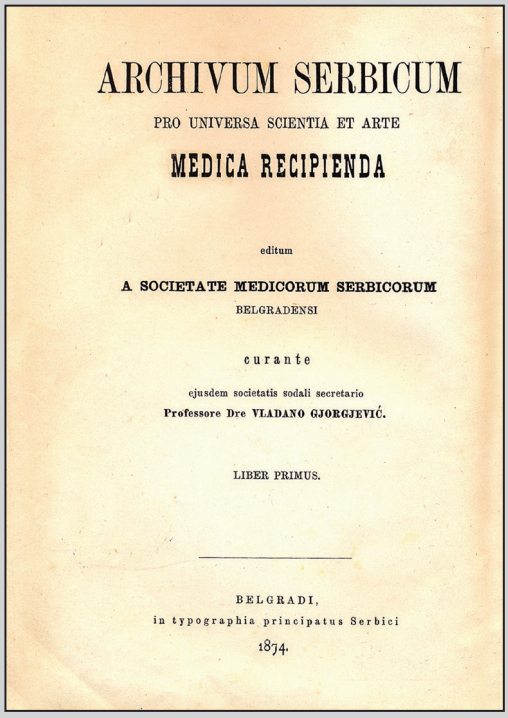
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of convalescent plasma in the treatment of severe acute respiratory distress syndrome caused by COVID-19 infection

Mirka Lukić-Šarkanović[1,2], Nina Vico-Katanić[1,2], Milica Jerković[1,2], Radojka Jokšić-Mazinjanin[1,3], Neda Terzić[4], Ranko Zdravković[1,5]

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SUMMARY

Introduction/Objective Convalescent plasma (CP) has been used in the past to treat several infectious diseases. It was hypothesized that CP could have a positive impact on severely ill patients with COVID-19 infection. The aim of the study was to present the results of CP application in patients with severe acute respiratory distress syndrome (ARDS) caused by COVID-19 infection.

Methods This is an observational study of critically ill patients who received CP according to the National Protocol for the Treatment of COVID-19 Infection at the University Clinical Center of Vojvodina in Novi Sad, Serbia, in 2020. Clinical outcomes were monitored before and after CP administration.

Results A total of 14 patients with severe life-threatening COVID-19 infection were included in the study. The patients age ranged 53–79 years. Most of them had two or more comorbidities, and more than half of them had blood type A Rh positive. Prior to CP administration, all patients received antibiotic therapy for severe pneumonia, corticosteroids, and anticoagulant therapy. Twelve out of 14 patients (85.7%) required endotracheal intubation and mechanical ventilation of the lungs, while two patients were on non-invasive mechanical ventilation. CP was administered 2–13 days after the confirmed diagnosis of COVID-19. The PaO_2/FiO_2 ratio before CP administration ranged 49.5–78.6. Twelve patients (85.7%) died during the course of the study.

Conclusion The use of CP in cases of severe ARDS caused by COVID-19 infection does not impact survival or lead to other forms of clinical improvement.

Keywords: COVID-19; acute respiratory distress syndrome; convalescent plasma

INTRODUCTION

Coronaviruses were identified about 60 years ago, and since then, there have been three major epidemics caused by these viruses. The first was the severe acute respiratory syndrome (SARS) epidemic in 2003, followed by the Middle East Respiratory Distress (MERS-CoV) in 2012. The third and most severe epidemic caused by coronaviruses is attributed to the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a disease known as COVID-19. The early variants of the COVID-19 virus caused severe infection in 10–25% of the infected individuals, primarily leading to pneumonia, with a certain percentage complicated by acute respiratory distress syndrome (ARDS) and a mortality rate of 39–49% [1, 2].

Convalescent plasma (CP) has been used to treat several severe viral infections such as SARS, MERS, Ebola, and avian flu [3, 4]. Studies investigating the effect of CP therapy on these viruses have shown higher survival rates in patients receiving CP [5, 6, 7]. Based on this, it was assumed that CP could also be beneficial for COVID-19 infection. The use of CP for treating critically ill patients with severe COVID-19 infection was approved by the US Food and Drug Administration early in the pandemic, following the results of observational studies that demonstrated its safety and potential to improve outcomes [8, 9]. This therapy relies on providing neutralizing antibodies against the SARS-CoV-2 spike protein to patients with active infection [10]. Initially, the recommendation was to administer CP to critically ill patients with life-threatening conditions, such as dyspnea, high respiratory rate, low blood oxygen saturation, and low PaO_2/FiO_2 ratio [11]. However, later research on CP's application in these patients gave conflicting results, with some studies showing no significant impact on the disease course.

In our country, CP was recommended during the early stages of infection according to the National Protocol for the Treatment of COVID-19 Infection. This study presents our earliest experience with administering CP to patients with COVID-19. The goal of the study was to determine if providing CP to patients with severe ARDS caused by COVID-19 can alter the disease course, considering that all patients who

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received CP based on the National Protocol were in a severe ARDS condition.

METHODS

This study was conducted at the Clinic for Anesthesia, Intensive Care, and Pain Management, University Clinical Center of Vojvodina in Novi Sad, Serbia, between July 10, 2020, and August 20, 2020. The study included a group of 14 participants with confirmed COVID-19 infection who received convalescent plasma (CP) treatment.

Plasma donors

The criteria for plasma donors were as follows:
- age between 18 and 60 years
- body weight greater than 60 kg
- absence of comorbidities that are permanent contraindications for blood donation
- the donor had a confirmed diagnosis of COVID-19 and/or a positive result for SARS-CoV-2 test (nasopharyngeal swab)
- a negative result for SARS-CoV-2 test from the nasopharyngeal swab, and at least 14 days had passed since the resolution of all symptoms.

All donors were provided with information regarding the purpose of plasma donation, the safety of the procedure, and the potential occurrence of adverse reactions during the donation process, along with the methods of prevention and management. Written consent and agreement for the mentioned procedure were obtained from all donors. Each donor underwent a mandatory medical examination, and all of them had a normal physical examination.

Preparation of plasma

The process of preparing plasma for CP donors involved several steps and tests to ensure safety and compatibility. When donors first arrived, blood samples were taken to determine their ABO and Rh blood groups, and they underwent screening for irregular anti-erythrocyte antibodies. Serological tests were conducted for human immunodeficiency virus (HIV), hepatitis B virus antigen (HBs), hepatitis C virus (HCV), and syphilis. Additionally, nucleic acid testing (NAT) was performed for HBV, HCV, and HIV. Complete blood counts, complete biochemical analyses, total proteins, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin, cholesterol, triglycerides, blood glucose, creatinine, urea, serum iron, total IgG concentration, and protein electrophoresis were also evaluated. If the donor had a history of previous transfusions or if the donor was a female with a history of pregnancies, anti-HLA antibodies were also checked. During subsequent plasmapheresis procedures, samples were taken for serological tests for HIV, HBs, HCV, syphilis, and NAT. Screening for irregular anti-erythrocyte antibodies and anti-COVID-19 antibodies was also performed during each session. Every two months, complete blood counts and measurements of total proteins, albumins, and ALT were repeated, with additional biochemical analyses and protein electrophoresis performed as needed. In cases where ALT levels were elevated, AST and GGT levels were also measured, and donors were allowed to donate plasma as long as ALT levels did not exceed twice the upper normal limit. Total IgG concentration was determined every 5 donations. When the results were in accordance with the procedures for donor plasmapheresis, plasmapheresis was performed. The plasma collection process itself was carried out by an apheresis procedure, plasmapheresis, which implies the exfusion of the donor's blood, the separation and retention of a certain volume of plasma and the reinfusion of cellular components without compensating the removed volume. The procedure was performed on MSC+ 9000 devices (Haemonetics Corporation, Boston, Massachusetts, USA), blood component separators, using sets that are sterile and intended for single use, with the use of anticoagulants and preservatives. The volume of plasma collected from a single donor during a plasmapheresis procedure was approximately 600 mL, and the plasmapheresis process took around 40 minutes. The collected plasma volume could not exceed 16% of the estimated total blood volume, which was calculated based on the donor's sex, height, and body weight or alternatively as 10 mL/kg of body weight.

Donors could contribute plasma multiple times, and the usual interval between two procedures was 15 days. The frequency of plasma donations depended on the concentration of IgG. For IgG concentrations ranging 6–8 g/L, donations were made every two weeks; for concentrations 8–10 g/L, donations were made weekly, and for concentrations above 10 g/L, donations were more frequent, but with a minimum interval of four days. If IgG concentration was less than 6 g/L, plasma donation was postponed for at least three weeks. The number of CP donations was also assessed by observing the presence of anti-COVID-19 antibodies. When a decrease in serologically detected antibodies was observed, plasma donation was stopped. For CP donors with no anti-COVID-19 antibodies detected by serological tests, plasma donation was limited to three months after the first donation. After collecting the unit of plasma (600 mL), it was divided into two equal parts of 300 mL by transferring the plasma into a transfer bag for plasma, using a sterile connection device. The original unit of plasma and the transfer bag were issued together. The storage process for the plasma was the same as for fresh-frozen plasma, with a storage duration of up to 36 months at a temperature of -25°C or lower. If the plasma was stored at a temperature between 20°C and 60°C, it could be stored for a maximum of 48 hours.

Plasma recipients

Each patient was assessed for CP administration based on a scoring system formulated by the transfusion specialists at the Clinical Center of Vojvodina, following the National protocol for the treatment of COVID-19 infection. ABO blood group compatibility with the donor was determined

Table 1. Demographic and clinical characteristics of patients who received convalescent plasma

Patient	Sex (m/f)	Age (years)	BMI kg/m^2	Comorbidities	Blood type	COVID-19 therapy	Invasive mechanical ventilation (yes/no)
1	m	53	38.5	obesity, HTN, DM, smoking, AF	B-	AB + C + DN	yes
2	m	70	27.8	HTN, smoking, angina pectoris	A+	AB + C + DN	yes
3	m	72	26.3	HTN	0-	AB + C + DN	yes
4	f	62	45.6	obesity, HTN, DM, asthma	A+	AB + C + DN	yes
5	m	72	24.8	HTN	A+	AB + C + DN	yes
6	m	66	23.3	smoking	0-	AB + C + DN	yes
7	m	54	34.1	obesity, HTN	B+	AB + C + DN	yes
8	m	79	32.8	obesity	A+	AB + C + DN	yes
9	m	72	39.1	obesity, HTN	B+	AB + C + DN	yes
10	m	70	41.5	obesity, HTN	A+	AB + C + DN	no
11	f	69	26.9	HTN, hypotireoidism	B-	AB + C + DN	yes
12	f	74	25.7	HTN	A+	AB + C + DN	yes
13	f	76	26.2	DM	A+	AB + C + DN	no
14	m	76	29.4	HTN, DM	A+	AB + C + DN	yes

m – male; f – female; BMI – body mass index; HTN – arterial hypertension; DM – diabetes mellitus; AF – atrial fibrillation; AB – antibiotic; C – corticosteroid – DN – dantrolen natrium

Table 2. Disease course

Patient	Number of days from confirmed diagnosis to CP transfusion	Number of days from ICU admission to CP transfusion	PaO_2/FiO_2			The outcome (died/survived)	Day of death/discharge after CP transfusion
			1 day before	1 day after	3 days after		
1	4	2	54.3	61.2	62.6	died	7
2	3	1	68.4	67.2	70.1	died	6
3	4	2	66.4	54.6	51.4	died	6
4	2	2	49.5	46.2		died	1
5	3	3	68.2	29.2		died	2
6	6	4	69.2	64.2		died	1
7	3	1	78.6	81.2		died	1
8	13	11	58.4	58.4		died	2
9	11	9	63.5	61	62.3	died	7
10	6	3	78.2	78.2	84.1	survived	15
11	6	6	70.1	67.2		died	0
12	3	1	64.6	68.4	67.8	died	19
13	5	3	76.3	78.6	82.2	survived	5
14	4	2	57.3	51.2	49.8	died	3

CP – convalescent plasma; ICU – intensive care unit

for each patient eligible for CP. All patients received two units of CP, each containing 300 ml, totaling 600 ml, on the same day. Clinical parameters for the patients before and after CP administration were obtained through a review of their hospital medical records and included the following data: basic demographic information, the number of days from confirmed COVID-19 diagnosis to CP transfusion, duration of hospitalization in the intensive care unit, presence of comorbidities, details of therapeutic modalities such as antibiotic, corticosteroid, and anticoagulant therapy, and the need for invasive mechanical ventilation. Laboratory parameters were monitored before and after CP administration for all patients. These parameters included arterial blood gas analysis, hemoglobin levels, hematocrit levels, leukocyte counts, platelet counts, D-dimer levels, fibrinogen levels, C-reactive protein (CRP) levels, procalcitonin (PCT) levels, and the Sequential Organ Failure Assessment (SOFA) score for each patient one day before CP administration, one day after, and three days after for those who survived beyond three days following CP administration.

Statistical analysis

Statistical processing of the obtained data was performed using the IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA) software package. The paired-sample t-test was used for statistical processing. Results are presented as means ± standard deviations (SD). Statistical significance was established at the $p < 0.05$ level.

Ethical approval for the study was obtained from the Ethics Committee of the University Clinical Center of Vojvodina in Novi Sad (00-51, 25/02/2020).

RESULTS

There were a total of 14 patients with severe ARDS caused by COVID-19 infection included in the study. The age of the patients was between 53 and 79 years (mean 68.9 ± 7.8 years) (Table 1). The average body mass index was 31.6 ± 7.1 kg/m^2. The majority were male (71.4%).

Table 3. Comparison of laboratory results before and after convalescent plasma transfusion

Patient	Hemoglobin (g/l)			Hematocrit (%)			WBC ($\times 10^9$/L)			Neutrophils (%)			Lymphocytes (%)			Platelets ($\times 10^9$/L)		
1	114	111	112	35.9	35.9	36.1	7.8	9.6	11.2	88.6	90.3	88.2	7.3	6.4	7.1	187	224	233
2	151	163	143	47	50.1	45.8	8.5	13.3	12.1	86.4	90.9	87.2	8.6	5.2	7.6	265	281	290
3	124	115	110	39.2	37	35.3	15.7	13.9	10.1	85.4	91.2	90.1	11.7	6.2	6.9	181	125	119
4	83	79		31.2	31.2		8.3	2.1		84.5	62.2		11.2	32.4		392	518	
5	124	102		36	31		17.7	18.6		86.6	88.9		11.1	8.3		220	210	
6	107	102		30.2	28.8		11.8	13.8		90.5	92.7		7.7	6		215	176	
7	113	112		35.4	35.2		13.5	15.7		94	95.2		4.4	3.1		334	137	
8	132	129		37	34		18.7	14.6		85.1	88.1		10.8	8.6		232	212	
9	141	128	131	45.9	39.8	40.2	18.9	11.2	10.2	94.9	89.9	87.2	1.5	3.9	4.2	125	229	170
10	127	123	121	40.8	38.4	38.1	5.4	6.2	6.4	89.7	88.8	88	7	7.8	7.1	323	354	366
11	82	50		25.9	17.8		32.4	27.9		14.3	14.3		84.7	84.7		64	64	
12	135	117	121	41.7	35.8	36.8	4.3	6.6	7.1	86.1	90.7	87.2	9.8	5.9	8.9	168	158	167
13	124	123	122	40.1	39.9	39.5	5.3	4.9	5.6	89.9	90.7	84.2	4.8	5.8	8.4	214	198	202
14	117	90	104	34.8	27.9	29.8	20.6	13.75	10.1	84	88.4	86.1	8.4	6.5	7.2	498	406	399

WBC – white blood cells

Table 4. Comparison of inflammatory and thrombogenesis markers – and SOFA score before and after convalescent plasma transfusion

Patient	D-dimer (ng/mL)		Fibrinogen (g/L)			CRP (mg/L)			Procalcitonin (ng/mL)			SOFA score		
1	385	403	4.2	3.9	3.4	92.8	68	72.2	0.06	0.12	0.56	6	5	7
2	1427	1398	4.9	3.5	3.3	6	23.3	37.4	0.12	0.21	0.77	4	4	5
3	1247	1298	3.1	3	3.2	187.6	277.8	262.1	0.18	0.21	0.48	5	6	7
4	759		3.2	3.7		23.3	54.7		0.21	0.29		4	4	
5	198		5.8	4.1		118.5	109.2		0.18	0.78		4	4	
6	348		3.2			75.2	182.3		7.04	9.12		6	6	
7	1120		8	4.4		138.6	131.6		1.02	1.42		4	3	
8	854		4.6	4.2		123.4	142.3		0.015	0.33		5	5	
9	675	687	4.1	3.8	3.5	212.2	187.3	192.2	6.37	6.15	7.23	5	5	7
10	882	839	3.1	2.3	2.4	375.6	178.2	201.2	0.44	0.28	0.65	4	4	3
11	1023	1112	4.9	4.6		154.7	204.7		0.28	0.31		7	7	
12	1186	987	3.5	3.5	3.7	61.4	59.4	63.2	0.48	0.71	0.92	5	4	5
13	289	322	3.2	4.2	3.8	203.4	156.2	188.3	0.21	0.08	0.12	4	4	3
14	156	207	4.4	4.1	3.2	506	327	366	0.98	1.1	1.2	5	6	7

CRP – C-reactive protein; SOFA score – Sequential Organ Failure Assessment score

Most of them had two or more comorbidities (78.6%). The most prevalent comorbidity was arterial hypertension (78.6%), followed by obesity (42.8%) and diabetes mellitus (28.6%). More than half of them had blood type A Rh positive (57.1%). Before receiving CP, all patients were receiving antibiotic therapy for severe pneumonia, corticosteroids, and anticoagulant therapy. Twelve out of 14 patients (85.7%) were endotracheally intubated and on mechanical ventilation, while two patients were on non-invasive mechanical ventilation.

In Table 2, it can be seen that CP was administered 2–13 days after confirmed COVID-19 diagnosis (average 5.2 ± 3.2 days) and 3.6 ± 3 days after admission to the ICU. The PaO_2/FiO_2 ratio before CP administration ranged 49.5–78.6 (mean 66 ± 8.8), indicating that all patients had severe ARDS according to the Berlin definition [12]. Twelve patients (85.7%) died, all of whom were on invasive mechanical ventilation. The mean PaO_2/FiO_2 ratio the day after CP administration was 62 ± 14, and it did not significantly differ ($p = 0.199$), nor three days later (PaO_2/FiO_2 66.3 ± 12.6, $p = 0.955$).

Most patients experienced a decrease in hemoglobin ($p = 0.002$) and hematocrit ($p = 0.007$) values after CP administration (Table 3). The values of leukocytes (neutrophilic granulocytes and lymphocytes) and platelets did not show a consistent trend of change.

Table 4 shows the values of D-dimer, fibrinogen, CRP, PCT, and SOFA score. The average D-dimer before CP administration was 724.4 ± 469.1 ng/mL, fibrinogen 4.3 ± 1.6, CRP 162.8 ± 135.5, PCT 1.2 ± 2.3, while the average SOFA score was 4.8 ± 0.9. The only value that significantly changed three days after CP administration was PCT, which was significantly higher ($p = 0.007$).

DISCUSSION

The idea of using CP for the treatment of COVID-19 infection emerged at the beginning of the pandemic [13]. This form of passive immunization has been used in the past for treating several infectious diseases, with varying degrees of success. In this case series, 14 patients with severe ARDS due to COVID-19 infection received CP. All patients received two doses of CP within 24 hours, following the National Protocol for the Treatment of COVID-19 Infection. What is interesting is that, upon meeting the

criteria for obtaining CP, all patients were actually in severe ARDS (PaO_2/FiO_2 < 100 mmHg). Upon reviewing the literature, no other case series with severe ARDS and CP treatment were found, making this study unique in its presentation of CP administration in severe ARDS cases. The patients age ranged 53–79 years, and the most common comorbidities were arterial hypertension, obesity, and diabetes mellitus, consistent with existing literature data that these are the most common conditions that lead to more severe forms of the disease [14–17].

Transfusion of plasma can lead to adverse events such as allergic reactions, febrile reactions, transmission of infection, hemolytic reactions, transfusion-related acute lung injury, and transfusion-associated circulatory overload [17]. In this study, most patients tolerated the transfusion well. Statistically significant changes were the decrease in hemoglobin and hematocrit values after CP administration, as well as an increase in PCT levels.

CP has been the most intensively studied treatment against COVID-19, and nearly 50 randomized controlled trials have provided evidence to assess its correct place in the anti-COVID-19 therapeutic armamentarium [18]. The first report of CP administration in a COVID-19 patient was published in March 2020 by Chinese authors Shen et al. [19]. Five critically ill patients received two doses of CP from healthy donors who had recovered from the infection. Four patients showed reductions in inflammatory biomarkers, improved gas exchange, and resolution of lung lesions based on computed tomography scans. However, the analysis of this study revealed that the patients were not in severe ARDS, unlike the patients in our study. A study by Habtehyimer et al. [20] also showed that CP administration led to a decreasing of inflammatory cytokines. Another study by Duan et al. [21] showed that the administration of one dose of CP was associated with improved clinical outcomes. However, a large randomized trial (RECOVERY trial) involving over 16,000 patients demonstrated that CP administration did not affect the disease course compared to standard therapy [22]. The results of this study align with the conclusions drawn by Zhang et al. [23] that CP transfusion in the later stages of the disease does not affect survival. This conclusion was also supported by a study conducted by Iranian scientists [24]. On the other hand, Misset et al. [25] showed in their study that CP therapy within five days of starting invasive mechanical ventilation reduced 28-day mortality.

A multicenter randomized study evaluated the cost-effectiveness of CP in patients diagnosed with COVID-19 from the Canadian public healthcare payer's perspective [26]. When compared to standard care, CP was more costly and less effective at improving quality-adjusted survival.

As can be seen from the discussion, the results of the therapy of COVID-19 infection with CP are not uniform and are certainly still a subject of controversy. The results of this study suggest that CP therapy was considered a last resort at that time, used when all other measures available at the time did not give results. It's important to note that these patients were treated during the first wave of COVID-19 infection in the country, around mid-2020, when randomized studies confirming the justification for CP administration were not yet available.

The study has some limitations. It is a case series with a relatively small number of patients and lacks a control group. All included patients had severe ARDS, so conclusions cannot be drawn for mild or moderate forms of ARDS. Potential complications of plasma transfusion may have been masked by the patient's severe general condition.

CONCLUSION

Based on our study we can conclude that the administration of CP in cases of severe ARDS caused by COVID-19 infection does not impact survival or lead to other forms of clinical improvement.

Conflict of interest: None declared.

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Ефекат конвалесцентне плазме у лечењу синдрома акутног респираторног дистреса узрокованог инфекцијом ковидом 19

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САЖЕТАК

Увод/Циљ Конвалесцентна плазма (КП) у прошлости се користила за лечење неколико инфективних болести. У складу са тим дошло се до хипотезе да би примена КП могла позитивно утицати на болеснике тешко оболеле од инфекције ковидом 19.

Циљ студије био је да прикаже резултате примене КП код болесника са тешком формом синдрома акутног респираторног дистреса узрокованог инфекцијом ковидом 19.

Методе Ово је серија случајева критично оболелих болесника којима је ординирана КП према Националном протоколу за лечење инфекције ковидом 19, у Универзитетском клиничком центру Војводине у Новом Саду (Србија), у 2020. години. Клинички исходи су праћени пре и после примене КП.

Резултати У студију је укључено 14 болесника са тешком, животно угрожавајућом инфекцијом ковидом 19. Старост болесника била је између 53 и 79 година. Већина је имала две или више придружених болести. Више од половине њих је било са *A Rh* позитивном крвном групом. Сви су, пре примене КП, добијали антибиотску терапију због тешке пнеумоније, кортикостероиде и антикоагулантну терапију. Дванаест од 14 болесника (85,7%) било је ендотрахеално интубирано и на механичкој вентилацији плућа, док су два болесника била на неинвазивној механичкој вентилацији. КП је ординирана у периоду 2–13 дана после потврђене дијагнозе ковида 19. Однос PaO_2/FiO_2 пре примене КП износио је 49,5–78,6. Преминуло је 12 болесника (85,7%).

Закључак Примена КП у стању синдрома тешког акутног респираторног дистреса узрокованог инфекцијом ковидом 19 не утиче на преживљавање нити на други вид клиничког побољшања.

Кључне речи: ковид 19; синдром акутног респираторног дистреса; конвалесцентна плазма

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effectiveness of the first and the second dose of COVID-19 vaccines in Serbia during the first three months of rollout

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SUMMARY

Introduction/Objective The main aim of this study was to assess COVID-19 vaccination effectiveness (VE) of BBIBP-CorV, Gam-COVID-Vac, BNT162b2, and ChAdOx1-nCoV-19 in Serbia during the first three months of rollout.

Methods The data from the Serbian National Immunization Registry, the Primary Health Centre Report, and the University Clinical Centre Report for Kragujevac, Serbia, for the period from January 1 to March 31, 2021 were used to compare COVID-19 vaccinated population to unvaccinated individuals in terms of laboratory confirmed SARS-CoV-2 infection, COVID-19-related hospitalization and intensive care unit (ICU) admission due to COVID-19. VE was estimated based on the incidence rate ratio, adjusted for age and sex.

Results Overall VE after the first dose reached 20.6%, 28.2%, and 56.1%, and 55.7%, 63.9%, and 79.8%, after the second dose for SARS-CoV-2 infection, COVID-19-related hospitalization, and ICU admission, respectively. BNT162b2 exhibited 96.7% VE against infection and no hospitalization after the second dose. Complete vaccination with BBIBP-CorV and Gam-COVID-Vac demonstrated VE of 43.2% and 78.6% against infection, 56.9% and 85.3% against hospitalization, and 82.3% and 52.7% against ICU admission, respectively. ChAdOx1-nCoV-19 after the first received dose showed VE of 10.3% and 74.7% against infection and hospitalization, with no ICU admission.

Conclusion COVID-19 vaccination in general, as well as each of the four studied vaccines, reduces the risk of SARS-CoV-2 infection, hospitalization due to COVID-19, and COVID-19-related ICU admission. Vaccine effectiveness significantly increases with the second received dose for all study outcomes.

Keywords: vaccine effectiveness; BBIBP-CorV; Gam-COVID-Vac; BNT162b2; ChAdOx1-nCoV-19

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INTRODUCTION

Since the outbreak of coronavirus disease 2019 (COVID-19), many scientific research groups and pharmaceutical companies worldwide joined their knowledge and efforts, giving rise to over 300 vaccines and vaccine candidates [1]. In Serbia, vaccination campaign started at the end of December 2020, with four different vaccines readily available [2]. Yet, initially rapid vaccine rollout, ranking Serbia first worldwide in terms of share of people vaccinated against COVID-19, has been reduced to worryngly low vaccination rate of only about 47% in January of 2022 (https://ourworldindata.org/covid-vaccinations). Public distrust of vaccines was deemed to be among the major reasons for this decline [3], warranting additional country-based investigations.

Phase 3 clinical trials have already shown that the efficacy in preventing COVID-19 of the vaccines in question clearly exceeds the threshold of 50% [4–7], set by the WHO [8]. However, due to constrained study populations, rigid criteria, and narrow range of possible outcomes, these conclusions may not reflect the real-world setting. On the other hand, vaccine effectiveness (VE), defined as an ability

to reduce the risk of infection, development of disease, or any other unwanted disease outcome in real-world conditions [8], has been recognized as very useful in assessing the actual COVID-19 vaccine performance. Yet, the reports on effectiveness of COVID-19 vaccines, although numerous, are usually limited to only certain vaccines, countries, and populations, considered only some of the relevant endpoints, or reported only a few of the important measures of effect [9, 10, 11].

Although recent meta-analyses indicate that VE generally decreases over time [12, 13], in the present study we aimed to assess the initial VE, as well as other indicators of short-term vaccine performance [including vaccine-preventable disease incidence (VPDI), and the number of subjects to be vaccinated to prevent one episode of COVID-19-related adverse outcome (NNV)], of four different COVID-19 vaccines during first three months of vaccine rollout in Serbia.

METHODS

Study design

This retrospective comparative cohort study was based on the data from the Serbian National Immunization Registry for the City of Kragujevac, and the Kragujevac Primary Health Centre and the Kragujevac University Clinical Centre Reports.

Registry data were used to assess COVID-19 vaccination coverage of population older than 16 years of age in Kragujevac, Serbia, between January 1 and March 31, 2021. Vaccinated subjects were considered those who received at least one dose of any of the four different COVID-19 vaccines available in Serbia at the time of the study, i.e. RNA-based BNT162b2 (Comirnaty®, Pfizer–BioNTech; New York, NY, USA; Mainz, Germany), inactivated BBIBP-CorV (Vero Cell®, Sinopharm Group Co. Ltd., Hong Kong, China), and vector-based Gam-COVID-Vac (Sputnik V®, Gamaleya National Center of Epidemiology and Microbiology, Moscow, Russia), and ChAdOx1-nCoV-19 (Vaxzevria®, University of Oxford/AstraZeneca; Oxford, UK; Cambridge, UK). Completely vaccinated (i.e. revaccinated) were considered those who received two doses of the same vaccine administered as recommended by the guidelines.

Reports data were explored for retrieving the information on unvaccinated subjects, assessing reverse transcription polymerase chain reaction (RT-PCR) or antigen test-confirmed cases of SARS-CoV-2 infection, and detecting the number of COVID-19 patients requiring hospitalization or intensive care unit (ICU) admission, registered within the same population between January 1 and May 3, 2021 (i.e. six weeks after the vaccination or revaccination of the last included vaccinated subject). RT-PCR or antigen test confirming SARS-CoV-2 infection was considered the primary outcome of the study, while the secondary outcomes included hospitalization due to COVID-19 and ICU admission. During the study period, 20I/Alpha was the predominant SARS-CoV-2 strain in Serbia [14].

The crude COVID-19 attack rate in Serbia, calculated for the period of three months preceding the study based on a cumulative number of confirmed COVID-19 cases in Serbia (https://ourworldindata.org/), was used for estimation of the minimum sample size for the study, according to the recommendations by the WHO [1].

Key eligibility criteria and follow-up schedule

Vaccinated subjects were included in the cohort if the data on sex, age, vaccination status, time and type of vaccine, and COVID-19 test result (if tested) were available, and if they had not been infected with SARS-CoV-2 prior to, or six weeks after vaccination (Supplementary Figure S1). COVID-19 cases tested positive within one week after receiving the first dose were excluded from the analysis [8].

The minimum sample size for the study, based on the crude COVID-19 attack rate in Serbia of 3.5% at the time of calculation, and assuming VE of 50%, precision of ± 10%, and type 1 error rate (α) of 0.05, was estimated to 8148 subjects. To achieve greater precision (since follow-up of vaccinated population longer than six weeks was not feasible), sample size has been increased to all available eligible subjects at the time of the study. Namely, there were 38,454 subjects found in the Registry data that fulfilled all the inclusion criteria for the vaccinated cohort. Unvaccinated subjects were selected at random from the eligible population with no prior SARS-CoV-2 infection. Out of 126,049 subjects that composed eligible study base for the unvaccinated cohort, 76,908 were randomly selected (controlling for sex and age, with the size ratio of 2:1) to be included in the study.

In terms of SARS-CoV-2 infection, vaccinated and unvaccinated subjects were followed up individually to a maximum of 63 (42 if only one dose was administered) and 122 days, respectively, or until they had been diagnosed with COVID-19. In terms of hospitalization and ICU admission, all SARS-CoV-2 infected subjects were followed up during the clinical course of COVID-19.

The process of selection of study cohorts is presented in Supplementary Figure S1. The study was approved by the Ethics Committee of the University Clinical Centre and the Primary Health Centre, Kragujevac, Serbia (approvals No 01/20-405, No 01/20-497, and No 01-1148/1, obtained on April 3, 2020, May 5, 2020, and February 24, 2021, respectively), and conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Statistical methods

Statistical analyses were performed using IBM SPSS Statistics, Version 20.0 (IBM Corp., Armonk, NY, USA), and Stata Statistical Software, release 16 (StataCorp LLC, Texas, USA). The frequencies of SARS-CoV-2 infection and COVID-19-related hospitalization and ICU admission over time were presented as incidence rate (IR). To estimate overall VE against all outcomes, vaccinated subjects were compared to unvaccinated by calculating incidence

Table 1. SARS-CoV-2 infection in the study cohorts, and the measures of vaccine effectiveness adjusted for age and sex

Variable	SARS-CoV-2 infection		IRR (95% CI)	VE (95% CI)	VPDI (95% CI)
	n	IR			
Unvaccinated	**5070**	**204**	NA		
Vaccinated (at least 1 dose)	**932**	**158.8**	**0.794 (0.740–0.852)**	**20.6% (14.8–26%)**	**40.4 (28.7–52.1)**
BBIBP-CorV	738	168.8	0.891 (0.824–0.964)	10.9% (3.6–17.6%)	891.2 (964.2–823.8)
Gam-COVID-Vac	116	152.3	0.671 (0.558–0.807)	32.9% (19.3–44.2%)	74.7 (46.2–103.1)
BNT162b2	34	63.3	0.273 (0.195–0.382)	72.7% (61.8–80.5%)	168.6 (146.2–191)
ChAdOx1-nCoV-19	44	222.7	0.897 (0.666–1.209)	10.3% (-20.9–33.4%)	25.2 (-41.2–91.6)
Revaccinated (2 doses)	**389**	**87**	**0.443 (0.399–0.491)**	**55.7% (50.9–60.1%)**	**107.8 (97.4–118.2)**
BBIBP-CorV	350	104.8	0.568 (0.509–0.634)	43.2% (36.6–49.1%)	78.7 (66.2–91.1)
Gam-COVID-Vac	36	48.5	0.214 (0.154–0.297)	78.6% (70.3–84.6%)	178.3 (161.2–195.4)
BNT162b2	3	7.7	0.033 (0.011–0.103)	96.7% (89.7–98.9%)	223.7 (212.6–234.8)

IR – incidence rate (per 1000 person-years); IRR – incidence rate ratio; VE – vaccine effectiveness; VPDI – vaccine-preventable disease incidence (per 1000 person-years); NA – not applicable

Table 2. SARS-CoV-2 infection in the vaccinated cohort per three weeks period, and the measures of vaccine effectiveness adjusted for age and sex

Variable	SARS-CoV-2 infection		IRR (95% CI)	VE (95% CI)	VPDI (95% CI)
	n	IR			
Unvaccinated	**5070**	**204**	NA		
First three weeks after 1st dose	**410**	**70.5**	**0.354 (0.320–0.391)**	**64.6% (60.9–68%)**	**128.7 (120–137.6)**
BBIBP-CorV	287	66.4	0.351 (0.311–0.395)	64.9% (60.5–68.9%)	121.7 (112.1–131.3)
Gam-COVID-Vac	67	88.6	0.391 (0.307–0.497)	60.9% (50.3–69.3%)	138.4 (116.2–160.5)
BNT162b2	26	48.4	0.209 (0.142–0.307)	79.1% (69.3–85.8%)	183.4 (163.5–203.4)
ChAdOx1-nCoV-19	30	152.7	0.616 (0.430–0.882)	38.4% (11.8–57%)	95.1 (39.8–150.4)
First three weeks after 2nd dose	**211**	**36.3**	**0.180 (0.156–0.206)**	**82% (79.4–84.4%)**	**162.9 (155.3–170.4)**
BBIBP-CorV	186	43	0.225 (0.194–0.361)	77.5% (63.9–80.6%)	145.1 (136.6–153.6)
Gam-COVID-Vac	22	29.1	0.128 (0.084–0.195)	87.2% (80.5–91.6%)	197.8 (184.1–211.6)
BNT162b2	3	5.6	0.024 (0.008–0.075)	97.6% (92.5–99.2%)	226.3 (216.7–235.8)
Second three weeks after 2nd dose	**169**	**29**	**0.145 (0.124–0.169)**	**85.5% (83.1–87.6%)**	**170.0 (162.9–177.4)**
BBIBP-CorV	156	36	0.190 (0.163–0.224)	81% (77.6–83.7%)	152.2 (144–160.2)
Gam-COVID-Vac	13	17.2	0.076 (0.044–0.131)	92.4% (86.9–95.6%)	209.7 (198.3–221)
BNT162b2	0	0	ND	ND	231.8 (224.7–238.9)

IR – incidence rate (per 1000 person-years); IRR – incidence rate ratio; VE – vaccine effectiveness; VPDI – vaccine-preventable disease incidence (per 1000 person-years); NA – not applicable; ND – not determined [due to zero event count (no COVID-19-positive cases) in vaccinated subjects]

rate ratio (IRR), using Mantel–Haenszel method to adjust for age and sex. VE was estimated by subtracting IRR between vaccinated and unvaccinated subjects (expressed as percentage) from 100% [1]. VPDI was calculated as a difference between incidences of an outcome in vaccinated and unvaccinated subjects, and reported per 1000 person-years [15]. NNV, as a number of subjects to be vaccinated to prevent one episode of COVID-19, one COVID-19-related hospitalization, or admission to ICU, was calculated as 1000 divided by VPDI [16].

RESULTS

Study participants

Demographic characteristics and the total length of the follow-up for SARS-CoV-2 infection of all 115,362 subjects involved in the study are presented in Supplemental Table S1.

Measures of VE against SARS-CoV-2 infection

IR (per 1000 person-years) of COVID-19 cases was 195.4 in the whole cohort, 158.8 in those who received at least one dose of any vaccine, and 204 in unvaccinated subjects. The risk of SARS-CoV-2 infection was significantly lower among vaccinated subjects as compared to unvaccinated population. Overall, VE increased with the second dose, as well as with time during the follow-up. Comparison among vaccines revealed the highest VE in BNT162b2, followed by Gam-COVID-Vac. The distribution of vaccinated and unvaccinated subjects among confirmed cases of SARS-CoV-2 infection, and the measures of VE during the total length of the follow-up and per three-week periods are presented in Tables 1 and 2.

Measures of VE against COVID-19 hospitalization

Hospitalization due to COVID-19 was registered among all study subjects with IR of 27.6 per 1000 person-years, and with IRs of 22.5 and 28.8 per 1000 person-years among vaccinated and unvaccinated, respectively. COVID-19 vaccination significantly reduced the risk, with VE increasing

Table 3. COVID-19-related hospitalization in the study cohorts, and the measures of vaccine effectiveness adjusted for age and sex

Variable	COVID-19-related hospitalization		IRR (95% CI)	VE (95% CI)	VPDI (95% CI)
	n	IR			
Unvaccinated	**715**	**28.8**	NA		
Vaccinated (at least 1 dose)	**132**	**22.5**	**0.718 (0.597–0.864)**	**28.2% (13.6–40.3%)**	**9.2 (4.7–13.8)**
BBIBP-CorV	125	28.6	0.856 (0.709–1.035)	14.4% (-3.5–29.1%)	5.4 (0.3–11.1)
Gam-COVID-Vac	5	6.6	0.240 (0.100–0.578)	76.0% (42.2–90%)	20.1 (14.7–27)
BNT162b2	1	1.9	0.079 (0.011–0.557)	92.1% (44.3–98.9%)	21.9 (17.7–26)
ChAdOx1-nCoV-19	1	5.1	0.253 (0.036–1.792)	74.7% (-79.2–96.4%)	15.0 (04.9–25.2)
Revaccinated (2 doses)	**52**	**11.6**	**0.361 (0.272–0.478)**	**63.9% (52.2–72.8%)**	**20.9 (16.8–24.9)**
BBIBP-CorV	49	14.7	0.431 (0.323–0.575)	56.9% (42.5–67.7%)	19.9 (14.9–24.9)
Gam-COVID-Vac	3	4	0.147 (0.047–0.457)	85.3% (54.3–95.3%)	23.5 (18.4–28.5)
BNT162b2	0	0	ND	ND	24.2 (22.2–26.1)

IR – incidence rate (per 1000 person-years); IRR – incidence rate ratio; VE – vaccine effectiveness; VPDI – vaccine-preventable disease incidence (per 1000 person-years); NA – not applicable; ND – not determined [due to zero event count (no hospitalization) in vaccinated subjects]

Table 4. COVID-19-related hospitalization in the vaccinated cohort per three-week periods, and the measures of vaccine effectiveness adjusted for age and sex

Variable	COVID-19-related hospitalization		IRR (95% CI)	VE (95% CI)	VPDI (95% CI)
	n	IR			
Unvaccinated	**715**	**28.8**	**NA**		
First three weeks after 1st dose	**63**	**10.8**	**0.345 (0.267–0.446)**	**65.5% (55.4–73.3%)**	**20.9 (17.3–24.5)**
BBIBP-CorV	61	14.1	0.422 (0.325–0.547)	57.8% (45.3–67.5%)	19.9 (15.4–24.3)
Gam-COVID-Vac	1	1.3	0.048 (0.007–0.342)	95.2% (65.8–99.3%)	26.1 (22.8–29.5)
BNT162b2	0	0	ND	ND	23.7 (21.8–25.7)
ChAdOx1-nCoV-19	1	5.1	0.254 (0.036–1.800)	74.6% (-80.0–96.4%)	15 (4.8–25.2)
First three weeks after 2nd dose	**32**	**7.2**	**0.225 (0.158–0.320)**	**77.5% (68–84.2%)**	**25.3 (21.7–28.9)**
BBIBP-CorV	30	6.9	0.210 (0.146–0.300)	79% (70–85.4%)	27 (23.4–30.7)
Gam-COVID-Vac	2	2.6	0.097 (0.024–0.386)	90.3% (61.4–97.6%)	24.8 (20.6–29)
BNT162b2	0	0	ND	ND	23.7 (21.8–25.7)
Second three weeks after 2nd dose	**17**	**2.9**	**0.091 (0.056–0.148)**	**90.9% (85.2–94.4%)**	**28.8 (26.0–31.6)**
BBIBP-CorV	16	3.7	0.108 (0.066–0.178)	89.2% (82.2–93.4%)	30.3 (27.0–33.6)
Gam-COVID-Vac	1	1.3	0.048 (0.007–0.342)	90.3% (65.8–99.3%)	26.2 (22.8–29.5)
BNT162b2	0	0	ND	ND	23.7 (21.8–25.7)

IR – incidence rate (per 1000 person-years); IRR – incidence rate ratio; IRD – incidence rate difference (per year); VE – vaccine effectiveness; VPDI – vaccine-preventable disease incidence (per 1000 person-years); NA – not applicable; ND – not determined [due to zero event count (no hospitalization) in vaccinated subjects]

with both doses received and with time during the follow-up. BNT162b2 and Gam-COVID-Vac exhibited the highest VE against hospitalization. The distribution of vaccinated and unvaccinated subjects among hospitalized cases of SARS-CoV-2 infection, and the measures of VE during the total length of the follow-up and per three week-periods are presented in Tables 3 and 4.

Measures of VE against COVID-19-related ICU admission

COVID-19-related ICU admission was registered among all study subjects with IR of 8.3 per 1000 person-years. COVID-19 vaccination significantly reduced the risk of COVID-19-related ICU admission: among unvaccinated, IR was 9.2 per 1000 person-years, as compared to the vaccinated cohort, with IR of 4.6 per 1000 person-years. VE increased with the second received dose and with time during the follow-up, and the higher VE was associated with BNT162b2, ChAdOx1-nCoV-19, and BBIBP-CorV. The distribution of vaccinated and unvaccinated subjects among SARS-CoV-2-infected admitted to ICU, and the measures of VE during the total length of the follow-up and per three-week periods are presented in Tables 5 and 6. Figure 1 presents NNV values for all three investigated COVID-19 outcomes among all vaccinated subjects, as well as per vaccine type in vaccinated with at least one, or with two doses of vaccine.

DISCUSSION

In the present study, we assessed the effectiveness of four different COVID-19 vaccines in terms of SARS-CoV-2 infection, hospitalization due to COVID-19, and COVID-19-related ICU admission. As to our best knowledge, this is the first time COVID-19 VE was investigated using the real-world data from Serbia. Our findings indicate that COVID-19 vaccination in general, as well as each of the investigated vaccines, significantly reduces the risk of all studied outcomes when compared to unvaccinated population. VE invariably increased with the second

Table 5. COVID-19-related intensive care unit (ICU) admission in the study cohorts, and the measures of vaccine effectiveness adjusted for age and sex

Variable	COVID-19-related ICU admission		IRR (95% CI)	VE (95% CI)	VPDI (95% CI)
	n	IR			
Unvaccinated	**229**	**9.2**	NA		
Vaccinated (at least 1 dose)	**27**	**4.6**	**0.439 (0.294–0.654)**	**56.1% (34.6–70.6%)**	**6 (3.7–8.2)**
BBIBP-CorV	22	5	0.441 (0.285–0.682)	55.9% (31.8–71.5%)	6.5 (3.9–9.2)
Gam-COVID-Vac	4	5.3	0.618 (0.230–1.658)	38.2% (-65.8–77%)	3.3 (2–8.6)
BNT162b2	1	1.9	0.264 (0.038–1.859)	73.6% (-85.9–96.2%)	5.3 (1.5–9.1)
ChAdOx1-nCoV-19	0	0	ND	ND	5.7 (4.7–6.7)
Revaccinated (2 doses)	**10**	**2.2**	**0.202 (0.106–0.382)**	**79.8% (61.8–89.4%)**	**8.7 (6.7–10.8)**
BBIBP-CorV	7	2.1	0.177 (0.083–0.376)	82.3% (62.4–91.7%)	9.8 (7.5–12.1)
Gam-COVID-Vac	3	4	0.473 (0.151–1.478)	52.7% (-47.8–84.9%)	4.5 (0.2–9.2)
BNT162b2	0	0	ND	ND	7.3 (6.3–8.3)

IR – incidence rate (per 1000 person-years); IRR – incidence rate ratio; VE – vaccine effectiveness; VPDI – vaccine-preventable disease incidence (per 1000 person-years); NA – not applicable; ND – not determined [due to zero event count (no admission to ICU) in vaccinated subjects]

Table 6. COVID-19-related intensive care unit (ICU) admission in the vaccinated cohort per three-week periods, and the measures of vaccine effectiveness adjusted for age and sex

	COVID-19-related ICU admission		IRR (95% CI)	VE (95% CI)	VPDI (95% CI)
	n	IR			
Unvaccinated	**229**	**9.2**	**NA**		
First three weeks after 1st dose	**12**	**2.1**	**0.197 (0.111–0.352)**	**80.3% (64.8–88.9%)**	**8.5 (6.7–10.4)**
BBIBP-CorV	12	2.8	0.244 (0.137–0.434)	75.6% (56.6–86.3%)	8.8 (6.6–11)
Gam-COVID-Vac	0	0	ND	ND	8.5 (7.4–9.7)
BNT162b2	0	0	ND	ND	7.1 (6.1–8.1)
ChAdOx1-nCoV-19	0	0	ND	ND	5.7 (4.7–6.7)
First three weeks after 2nd dose	**6**	**1.3**	**0.122 (0.054–0.276)**	**87.8% (72.4–94.6%)**	**9.6 (7.7–11.4)**
BBIBP-CorV	4	0.9	0.081 (0.030–0.216)	91.9% (78.4–97%)	10.7 (8.8–12.5)
Gam-COVID-Vac	2	2.7	0.316 (0.079–1.273)	68.4% (-27.3–92.1%)	5.8 (1.9–9.8)
BNT162b2	0	0	ND	ND	7.3 (6.3–8.3)
Second three weeks after 2nd dose	**3**	**0.7**	**0.059 (0.018–0.189)**	**94.1% (81.1–98.2%)**	**10.3 (8.6–12)**
BBIBP-CorV	2	0.6	0.048 (0.012–0.202)	95.2% (79.8–98.8%)	11.3 (9.4–12.2)
Gam-COVID-Vac	1	1.4	0.157 (0.022–1.128)	84.3% (-12.8–97.8%)	7.9 (4.3–10.1)
BNT162b2	0	0	ND	ND	7.3 (6.3–8.3)

IR – incidence rate (per 1000 person-years); IRR – incidence rate ratio; VE – vaccine effectiveness; VPDI – vaccine-preventable disease incidence (per 1000 person-years); ND – not determined [due to zero event count (no admission to ICU) in vaccinated subjects]

received dose, and the similar trend has been observed over the six-week-long follow up after complete vaccination. BNT162b2, followed by Gam-COVID-Vac demonstrated the highest VE against all outcomes of interest in terms of SARS-CoV-2 infection and hospitalization due to COVID-19, which was the case with ChAdOx1-nCoV-19 and BBIBP-CorV in terms of COVID-19-related ICU admission.

Since the 18th century, vaccination has been recognized as one of the most effective measures for reducing morbidity and mortality of infectious diseases [17]. Thus, it came as no surprise that "once in a century" pandemic such as COVID-19, after failing to succumb to intensive public health interventions, would raise high expectations for the vaccine. Once available, vaccination triggered both efficacy and effectiveness studies, where SARS-CoV-2 strong transmission ability and extremely unpredictable course of the disease placed infection rate, hospitalization, and ICU admission among the most important COVID-19-related outcomes. Numerous real-world-setting investigations have been conducted so far, and VE has been assessed in different countries on hundreds of thousands of subjects [10, 18, 19]. To the best of our knowledge, this study is the first to simultaneously investigate and report VE of BNT162b2, ChAdOx1-nCoV-19, Gam-COVID-Vac, and BBIBP-CorV for three different COVID-19-related outcomes in Serbia.

Previous studies were unified in conclusion that COVID-19 vaccination with BNT162b2 provides significant protection, which increases with time, and achieves its full potential after the second dose [20, 21, 22]. In terms of SARS-CoV-2 infection, hospitalization due to COVID-19 and COVID-19-related ICU, reported BNT162b2 VE in completely vaccinated subjects ranged from 65% [21] to 97% [9], from 80% to 98% [23], and from 90% [21] to 98% [9], respectively. In our study, BNT62b2 proved to have the highest VE in terms of all investigated outcomes, corresponding well to previously published data. Our study

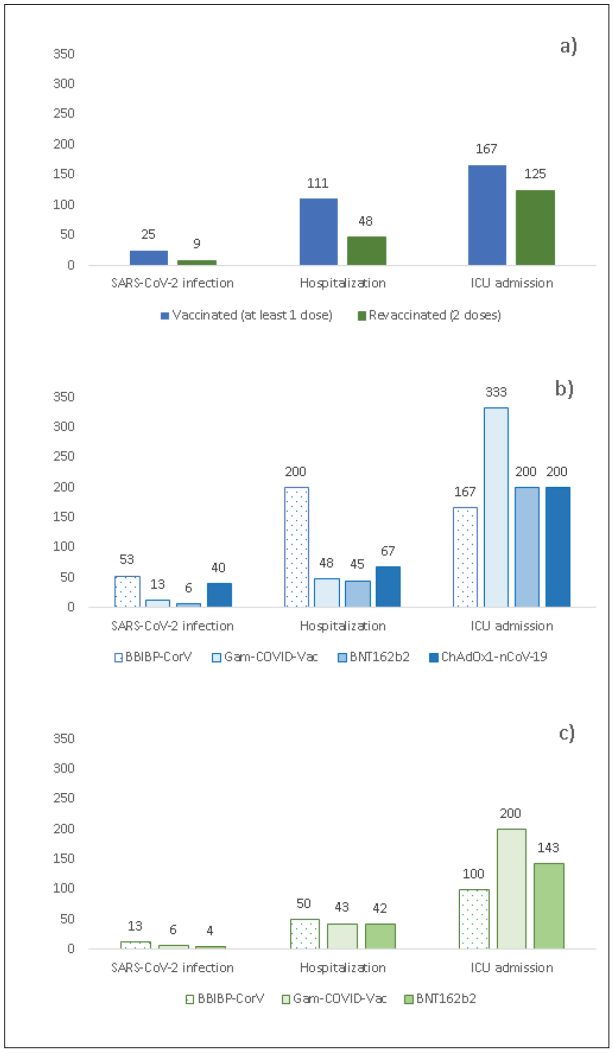

Figure 1. NNV (number of subjects to be vaccinated to prevent one episode of COVID-19, one COVID-19-related hospitalization, or admission to ICU) for three investigated COVID-19-related outcomes in a) all vaccinated subjects, b) vaccinated with at least one dose, and c) vaccinated with two doses

contributes to the existing evidence on BNT162b2 VE in two different ways: a) by providing the data from a population that has not been previously explored in this regard, and b) by simultaneously reporting VPDI and NNV values, which are recognized as important clinical indicators of the vaccination-driven COVID-19 risks reduction [24].

On the other hand, the effectiveness studies on ChAdOx1-nCoV-19 are scarce and mainly associated with VE after the first dose [11, 19, 20, 21]. In terms of SARS-CoV-2 infection and hospitalization due to COVID-19, ChAdOx1-nCoV-19 VE after one dose ranged from 36% [11] to 78% [21], and from 50% to 88% [19], respectively. Yet, none of the previous studies reported its VE for COVID-19-related ICU admission. Our data indicate that ChAdOx1-nCoV-19 in one dose modestly decreases the risk of infection, but significantly reduces the risk of hospitalization or ICU admission due to COVID-19. Limited by relatively short follow-up, we were not able to assess VE of ChAdOx1-nCoV-19 after revaccination, which remains to be elucidated in the future.

In spite of the worldwide deployment of Gam-COVID-Vac and BBIBP-CorV, we were able to find only a few published reports on their effectiveness against COVID-19-related outcomes [25]. In a Hungarian study by Voko et al. [26], Gam-COVID-Vac and BBIBP-CorV demonstrated VE against SARS-CoV-2 infection, assessed at least seven days after the second dose, of 85.7% and 68.7%, respectively. In Argentina, administration of one dose of Gam-COVID-Vac displayed VE of 78.6%, 87.6%, and 84.8% in preventing laboratory-confirmed infection, reducing hospitalizations, and deaths, respectively [27]. On the other hand, Zhang et al. [28] reported BBIBP-CorV VE against hospitalization for serious or critical illness in Morocco of 88.5%, while the data from the United Arab Emirates indicate effectiveness of the same vaccine against hospitalization, critical care admission, and death due to COVID-19 of 79.6%, 86%, and 84.1%, respectively [29]. In our study, in terms of SARS-CoV-2 infection these two vaccines proved to be slightly more effective when assessed three weeks after complete vaccination, and their effectiveness during the study follow-up increased with time. In addition, we have shown that both Gam-COVID-Vac and BBIBP-CorV are effective against two other investigated outcomes, namely hospitalization and ICU admission. They differed in terms of outcomes for which they were more effective, with Gam-COVID-Vac demonstrating higher VE in reducing the risk of hospitalization and the risk of infection, and BBIBP-CorV, the most frequently administered vaccine in Serbia, being more protective against COVID-19-related ICU admission.

It should be noted that this report has several limitations. Firstly, we were unable to assess the level of exposure to SARS-CoV-2 among vaccinated and unvaccinated subjects. The exposure risk can vary considerably, as it depends on the environment, health status, human behavior, and many other factors [30]. Since it enables viral transmission and significantly affects the initial infectious dose, the level of exposure to SARS-CoV-2 can be crucial for the development and the fate of COVID-19 [31]. Furthermore, certain factors associated with the risk of exposure could also limit the accuracy of our findings, leading to either under- or overestimation of VE. On one hand, there is the healthy adherer effect, which suggests that the vaccinated subjects should be more likely to practice preventive measures that decrease the risk of infection [32], hence attributing at least part of the observed effect to precautions rather than to vaccination. On the other hand, COVID-19 vaccination could trigger the so-called Peltzman effect, which implies that vaccinated individuals might feel more protected and thus get involved in riskier behavior [33], blurring the real VE. Also, we did not assess the symptoms of the infected subjects, so there is a possibility that asymptomatic people, who are generally less likely to be tested for SARS-CoV-2 infection were omitted from our study. Having in mind that the "silent" infections can comprise more than one third of all COVID-19 cases [34], and that their viral loads, as well as the risk of further disease transmission, can be comparable to symptomatic infections, it would be prudent to include them

too in the assessment of VE. Furthermore, our data were collected before the appearance of new Delta and Omicron variants of SARS-CoV-2 in Serbia [35], during only a six-week-long period, and before the third dose of vaccines was available, rendering our results less relevant to new strains of the virus, and missing out the information on the VE later during the period after complete vaccination, or after receiving more than two doses. Also, most of the vaccinated subjects received BBIBP-CorV, and that might potentially affect the results of comparison among different types of vaccines. Finally, our study included population of only one region in Serbia, which is mainly of Serbian origin. Since the susceptibility to SARS-CoV-2 infection and the severe form of the disease has been linked to polymorphism of certain genes [36], there is a possibility that the effect of vaccination in Serbs is under the influence of their ethnicity-related genetic signature, depreciating the applicability of our results to other populations.

CONCLUSION

In conclusion, our study, based on the real-world data from Serbia, demonstrates that COVID-19 vaccination in general, as well as each of the four studied vaccines, reduces the risk of SARS-CoV-2 infection, hospitalization due to COVID-19, and COVID-19-related ICU admission. VE significantly increases with the second received dose for all study outcomes.

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Ефективност прве и друге дозе вакцина против ковида 19 у Србији током прва три месеца од почетка вакцинације

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САЖЕТАК

Увод/Циљ Циљ студије је био да се процени ефективност вакцинације (ЕВ) против ковида 19 вакцинама *BBIBP-CorV*, *Gam-COVID-Vac*, *BNT162b2* и *ChAdOx1-nCoV-19* у Србији током прва три месеца од почетка вакцинације.

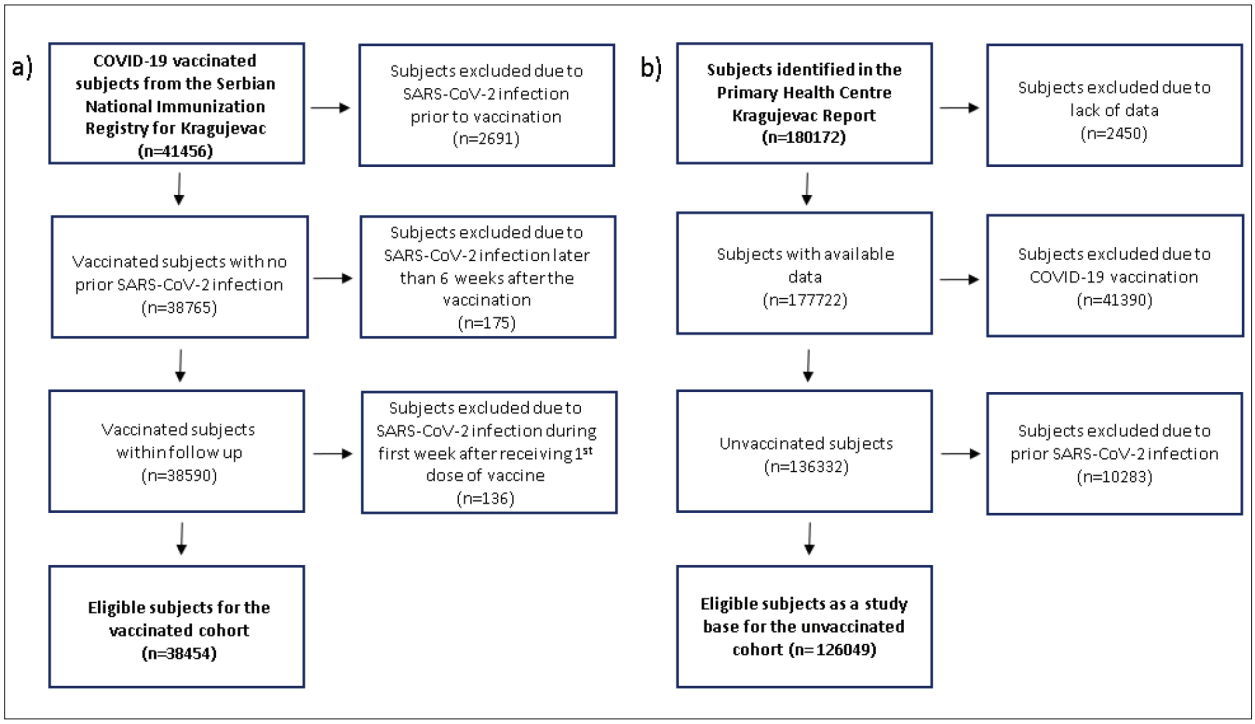
Методе Подаци за период од 1. јануара до 31. марта 2021. прикупљени из Националног регистра за имунизацију Србије, Извештаја Дома здравља и Извештаја Универзитетског клиничког центра „Крагујевац", Србија, коришћени су за поређење вакцинисане са невакцинисаном популацијом у погледу лабораторијски потврђене инфекције *SARS-CoV*-2, хоспитализације због ковида 19 и пријема у јединицу интензивне неге (ЈИН) због ковида 19. ЕВ је процењена на основу односа стопе инциденције, прилагођене за старост и пол.

Резултати Укупна ЕВ за све вакцине после прве дозе достигла је 20,6%, 28,2% и 56,1% у погледу инфекције *SARS-CoV*-2, хоспитализације због ковида 19 и пријема у ЈИН, а 55,7%, 63,9% и 79,8% после друге дозе за исте исходе. *BNT162b2* је достигла ЕВ од 96,7% против инфекције, и није било хоспитализације после друге дозе. Потпуна вакцинација вакцинама *BBIBP-CorV* и *ChAdOx1-nCoV-19* показала је ЕВ од 43,2% и 78,6% против инфекције, 56,9% и 85,3% против хоспитализације и 82,3% и 52,7% против пријема у ЈИН. *ChAdOx1-nCoV-19* је после прве дозе показала ЕВ од 10,3% и 74,7% против инфекције и хоспитализације, без пријема у ЈИН.

Закључак Вакцинација против ковида 19 уопште, као и свака појединачна вакцина, смањила је ризик од инфекције *SARS-CoV*-2, хоспитализације због ковида 19 и пријема у ЈИН због ковида 19. Ефикасност вакцине значајно се повећава са другом примљеном дозом за сва три исхода праћена у студији.

Кључне речи: ефективност вакцине; *BBIBP-CorV*; *Gam-COVID-Vac*; *BNT162b2*; *ChAdOx1-nCoV-19*

SUPPLEMENTARY MATERIAL



Supplementary Figure S1. The process of selection: a) the cohort of vaccinated subjects, and b) the study base for the cohort of unvaccinated subjects

Supplementary Table S1. Demographic characteristics, vaccination status, and the total length of the follow-up of subjects involved in the study

Characteristics	Vaccinated subjects		Unvaccinated subjects		Total	
	n	%	n	%	n	%
Total	38,454	33.33	76,908	66.67	115,362	100
Age groups (years)						
Up to 24	268	0.7	536	0.7	804	0.7
25–34	1309	3.4	2618	3.4	3927	3.4
35–44	4238	11.02	8476	11.02	12,714	11.02
45–54	5318	13.83	16,519	21.48	21,837	18.93
55–64	7994	20.79	15,897	20.67	23,891	20.71
65–74	13,030	33.88	16,823	21.87	29,853	25.88
75 and over	6297	16.38	16,039	20.85	22,336	19.36
Sex						
Male	20,534	53.4	41,044	53.37	61,578	53.38
Female	17,920	46.6	35,864	46.63	53,784	46.62
Vaccinated (at least 1 dose)	38,454	33.33	0	0	38,454	33.33
BBIBP-CorV	28,630	24.82	0	0	28,630	24.82
Gam-COVID-Vac	4522	3.92	0	0	4522	3.92
BNT162b2	3559	3.09	0	0	3559	3.09
ChAdOx1-nCoV-19	1743	1.51	0	0	1743	1.51
Vaccinated (only 1 dose)	12,477	10.82	0	0	12,477	10.82
BBIBP-CorV	9206	7.98	0	0	9206	7.98
Gam-COVID-Vac	223	0.19	0	0	223	0.19
BNT162b2	1305	1.13	0	0	1305	1.13
ChAdOx1-nCoV-19	1743	1.51	0	0	1743	1.51
Revaccinated (2 doses)	25,977	22.52	0	0	25,977	22.52
BBIBP-CorV	19,424	16.84	0	0	19,424	16.84
Gam-COVID-Vac	4299	3.73	0	0	4299	3.73
BNT162b2	2254	1.95	0	0	2254	1.95
Total person time						
In days	2,138,842		9,075,850		11,214,692	
In years	5,859.84		24,865.34		30,725.18	

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Impact of COVID-19 pandemic on changing the ratio of abdominal, vaginal, and laparoscopic hysterectomies

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SUMMARY
Introduction/Objective Coronavirus pandemic caused most hospitals in the world to suspend regular activities. The aim of this study was to analyze pandemic influence on patients who underwent hysterectomy with classical (abdominal) and minimally invasive surgery (laparoscopic and vaginal approach) at the Clinic for Gynecology and Obstetrics of the University Medical Centre of Serbia.
Methods There were 2446 hysterectomies for five-year period. The study analyzed number and types of hysterectomies before and during COVID-19 pandemic.
Results The total number of operated patients was most decreased in the first year of the pandemic. During pandemic, the number of vaginal and laparoscopic hysterectomies did not change, whereas there was an increase in the number of abdominal hysterectomies. Statistical significance ($p < 0.01$) was found between abdominal and vaginal as well as between abdominal and endoscopic hysterectomies.
Conclusion The global pandemic impact on care of symptomatic patients with COVID-19 has led to the redeployment of staff and resources, which has significantly reduced the total number of operations in many hospitals around the world.
Keywords: COVID-19; gynecological surgery; laparoscopy

INTRODUCTION

The epidemic caused by the SARS-CoV-2 began at the end of 2019, and in just a few months affected almost the entire world. The high morbidity and mortality induced by this virus caused problems in the health systems in many countries, and many hospitals suspended or significantly reduced their regular activities in order to engage medical staff for patients suffering from COVID-19 [1, 2]. Due to this emergency, the number of elective surgeries has been reduced.

Hysterectomy is one of the most frequent surgeries in the field of gynecology and represents a mainstay in management of various benign and malignant diseases. An abdominal, vaginal, laparoscopic or robotic approach can be utilized depending on numerous factors such as underlying pathology, shape, and size of the uterus, adnexal pathology, surgical risk and surgeon expertise [3].

In contemporary gynecological practice, minimally invasive surgery (MIS) is considered the technique of choice in most clinical scenarios, but concern has been raised that SARS-CoV-2 could be disseminated during such procedures and when using smoke-generating devices [4]. Hence, employing MIS during COVID-19 pandemic was deemed potentially hazardous by some experts [1].

The aim of this study was to analyze whether COVID-19 pandemic had influenced the number of patients referred for hysterectomy, and whether it had affected the surgical approach selection.

METHODS

In this retrospective cohort study, data were compiled from medical records and operative protocols of the Clinic for Gynecology and Obstetrics of the University Clinical Centre of Serbia. All patients who had undergone a hysterectomy from the beginning of 2017 to the end of 2021 were included in the study. The surgical approaches were also noted – total abdominal, vaginal, and laparoscopic hysterectomy. Both total laparoscopic hysterectomy (TLH) and laparoscopically-assisted vaginal hysterectomy (LAVH) were part of the laparoscopic hysterectomy group. We have analyzed the total number of hysterectomies per year and compared the number of hysterectomies in 2019 compared to 2020. Finally, we analyzed the average number of yearly hysterectomies between the pre-pandemic and pandemic years (2017–2019 *vs*. 2020–2021). We used IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp. Armonk, NY, USA) for statistical analysis. We chose a 0.05 level of statistical

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significance. Data were described by using ratios and percentages while discrete variables were compared using χ^2 and Fisher tests as appropriate.

The study was approved by Ethics Committee of the University Clinical Centre of Serbia (number 1038/1).

RESULTS

A total of 2446 hysterectomies were performed over a five-year period. Most of the hysterectomies (1865/2446, 76.2%) were done using the abdominal approach. A vaginal approach was used in 473 patients (19.3%), whereas laparoscopy was performed in 108 patients (4.4%) (Figure 1). The total number of hysterectomies per year by surgical approach are presented in Table 1.

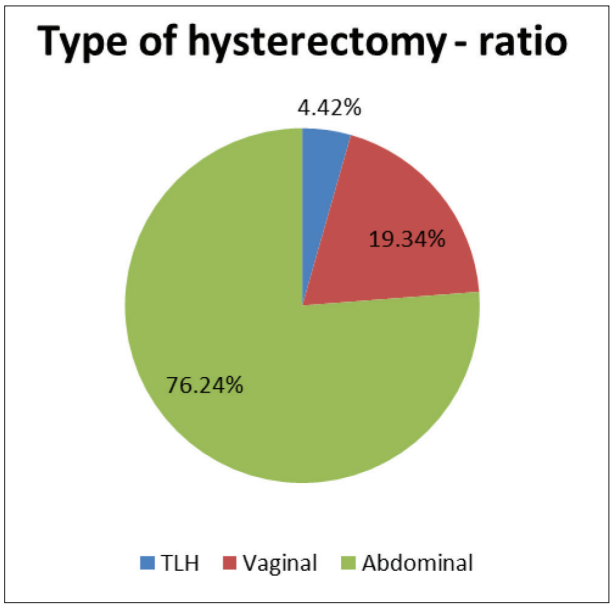

Figure 1. Total relative numbers according to the type of hysterectomy; TLH – total laparoscopic hysterectomy

Table 1. Numbers and types of hysterectomies per year

Year		2017	2018	2019	2020	2021	Total
Types of hysterectomy	TLH/LAVH	17	25	29	19	18	108
	VAG	177	126	92	39	39	473
	TAH	421	389	375	319	361	1865
Total		615	540	496	377	418	2446

TLH – total laparoscopic hysterectomy; LAVH – laparoscopically assisted-vaginal hysterectomy; VAG – vaginal hysterectomy; TAH – total abdominal hysterectomy

The highest number of hysterectomies was recorded in 2017, while the lowest was observed in the first year of the COVID-19 pandemic, i.e. 2020). Although there has been a steady decrease in the total number of hysterectomies per year from 2017 to 2020, the abdominal approach was still the most prevalent, followed by the vaginal, and laparoscopic approach. During the second year of the pandemic (2021), the number of hysterectomies increased but did not reach pre-COVID levels (Figure 2).

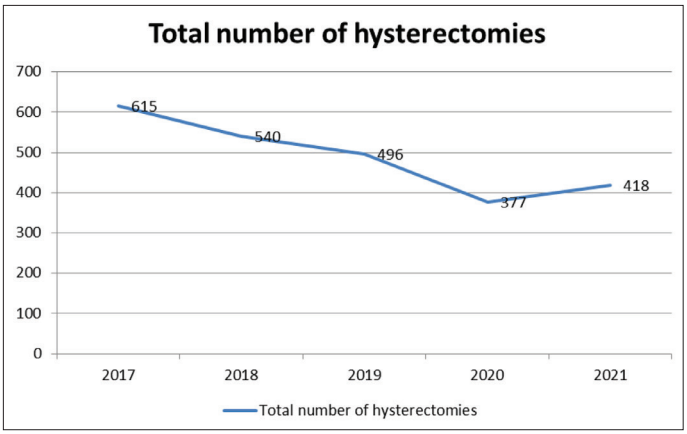

Figure 2. Total number of hysterectomies over the years

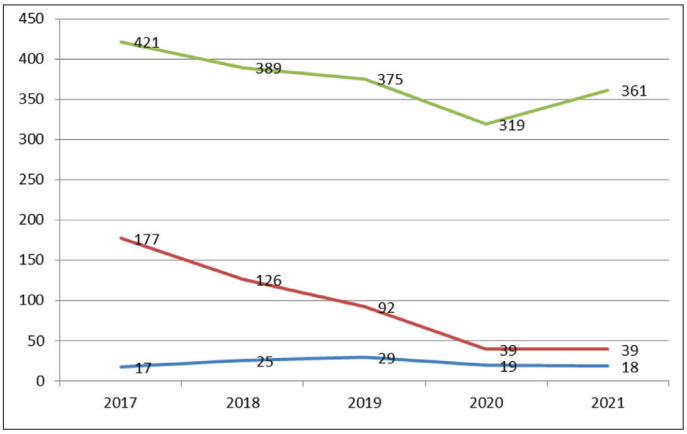

Figure 3. Number and type of hysterectomies during years; TLH – total laparoscopic hysterectomy; LAVH – laparoscopically-assisted vaginal hysterectomy

The relative change in the number of hysterectomies was most pronounced in the vaginal hysterectomy subgroup – approximately 70%. Decreases in the number of TLH/LAVH and abdominal hysterectomies can also be observed – 25% and 14% decreases, respectively (Figure 3). Also, when presented as relative numbers, it is noticeable that the total number of hysterectomies significantly dropped mostly because of the decreased number of vaginal and laparoscopic operations (Table 2 and Figure 4).

During the second year of pandemic a slight increase in the number of total abdominal hysterectomies was observed compared to the first pandemic year, whereas the number of vaginal and TLH/LAVH did not change (Figure 3).

When a χ^2 test was used to compare the number of hysterectomies by each approach between the year 2019 and the year 2020, a highly statistically significant difference ($\chi^2 = 12.05$, $p = 0.002$) was observed. The percentage of vaginal hysterectomies accounted for 18.5% of all hysterectomies completed in 2019, while the same percentage was 10.3% in 2020. Conversely, 75.6% of all hysterectomies were total abdominal hysterectomies in 2019 but 85.4% in 2020.

When pre-pandemic years (2017–2019) were compared to pandemic ones (2020–2021), similar conclusions to the ones outlined in the previous paragraph could be drawn.

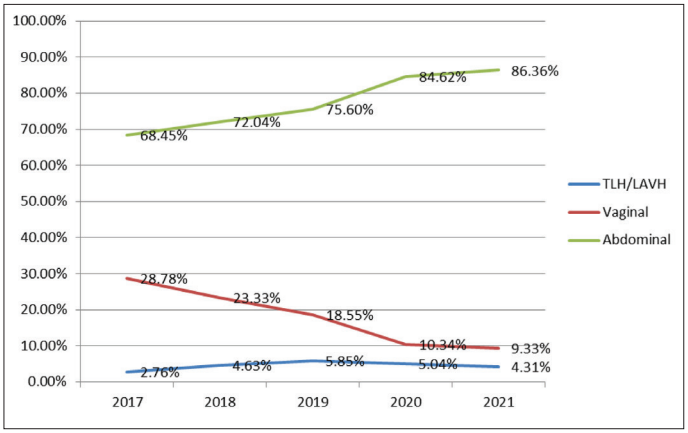

Figure 4. Relative numbers and types of hysterectomies over time; TLH – total laparoscopic hysterectomy; LAVH – laparoscopically-assisted vaginal hysterectomy

Table 2. Relative numbers and types of hysterectomies per year

Procedure	2017	2018	2019	2020	2021	Total
TLH/LAVH	2.76%	4.63%	5.85%	5.04%	4.31%	4.42%
VAG	28.78%	23.33%	18.55%	10.34%	9.33%	19.34%
TAH	68.45%	72.04%	75.60%	84.62%	86.36%	76.24%
						100%

TLH – total laparoscopic hysterectomy; LAVH – laparoscopically-assisted vaginal hysterectomy; VAG – vaginal hysterectomy; TAH – total abdominal hysterectomy

A very highly statistically significant difference ($p < 0.001$) was observed between these two groups with a smaller relative contribution of vaginal hysterectomies (9.8% *vs.* 23.9%) and a larger relative contribution of total abdominal hysterectomies (85.5% *vs.* 71.8%) in pandemic years compared to pre-pandemic ones respectively.

DISCUSSION

There was a 28% drop in the total number of hysterectomies performed at our institution over a five-year period, with the largest decrease in vaginal hysterectomies.

Although studies comparing surgical approach before and during pandemic are scarce, two studies analyzed impact of COVID-19 pandemic on gynecological surgery department. Piketty et al. [5] reported a 65% decrease of operations and surgical interventions during COVID-19 lockdown in one of Paris's gynecological departments, whereas Gupta et al. [6] reported an even higher reduction of approximately to 75%. Hence, the total reduction in our clinic was less than the one observed in developed countries and, even though not statistically significant, the increase in the number of operations during second year of pandemic implied that our health care system managed to organize activities in the extreme circumstances.

The impact of the pandemic led to the redeployment of the staff and resources due to the treatment of symptomatic patients with COVID-19, which had a significant impact on the reduction of the total number of operations in many hospitals around the world, which was mostly reflected in reduced number of elective non-emergent, vaginal and minimally invasive procedures [7]. This is in accordance with our results which showed the highest drop for vaginal hysterectomies. The surgery organization was adapted to include emergency and oncological cases only. Although COVID-19 pandemic could have posed a risk in time delaying from symptom onset to intervention, retrospective studies showed no difference in [8]. On the other hand, non-emergency surgeries were significantly reduced during the first year of pandemic. Data from the National Inpatient Sample and the National Ambulatory Surgery Sample included 1,029,792 hysterectomies performed in the USA during 2019, while that number greatly decreased in 2020; The greatest decrease was observed from March to May of 2020, corresponding with the initial wave of COVID-19 [9].

Vaginal and minimally invasive surgical procedures are certainly the best choice for patients, but there are objective reasons why they are performed less than expected in pandemic settings. Firstly, it is necessary to have appropriate equipment and trained personnel at your disposal. Also, one needs to properly select patients in who MIS will provide good results. Suspicion of ovarian malignancy, adnexal masses larger than 10 cm, larger pelvic tumors, scars and adhesions from previous operations represent some limitations for the laparoscopic approach [10, 11]. The training and experience of the surgical and anesthesiology teams are also important factors influencing the ratio of the number of abdominal and laparoscopic hysterectomies [12].

Also, due to the high incidence of COVID-19 in the general population, the possibility of dispersal of infected droplets and aerosols during laparoscopic surgery has once again become a topic of discussion in scientific circles [13]. Laparoscopy involves creating a pneumoperitoneum with carbon-dioxide insufflation and previously studies have demonstrated the presence of viral DNA such as that of hepatitis B virus and human papillomavirus in surgical smoke [14]. Thus, the aerosol could potentially be contaminated with the SARS-CoV-2 virus due to even minimal leakage of CO_2, as well as smoke generated during energy devices use, leading additionally to a temporary shift in favor of open surgery [7].

On the other hand, a systematic review by Matta et al. [15] on COVID-19 transmission via surgical smoke during laparoscopy found no cases of viral transmission in the operating theatre. However, a potential risk exists, and caution should be exercised while further investigations are conducted.

The pandemic also brought up potential socio-demographic problems. One American study showed significant difference in the decline in the number of hysterectomies among different races, which showed how hospitals prioritized certain gynecologic surgeries as elective [16].

Additionally, postponing scheduled operations and the fear of contracting SARS-CoV-2 in hospitals may lead to significant anxiety according to a survey including 16 European countries [15]. Also, a day case hysterectomy has been successfully proposed in order not to delay elective surgery as a solution due to redistribution of staff and capacity of hospitals [17].

CONCLUSION

The COVID-19 pandemic decreased the total number of hysterectomies at our clinic. The number of vaginal and laparoscopic hysterectomies compared to classical, total abdominal hysterectomies was significantly reduced in Serbia, as well as all around the world, due to the enormous modifications of health care systems. Surgery postponement and consequences caused by this shift regarding progression of primary disease, survival rate and quality of life are yet to be investigated.

Conflict of interest: None declared.

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Утицај пандемије ковида 19 на промену односа броја абдоминалних, вагиналних и лапароскопских хистеректомија

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САЖЕТАК

Увод Пандемија изазвана вирусом корона довела је до тога да већина болница у свету обустави или значајно смањи редовне активности.

Циљ рада је био да се анализира утицај пандемије на однос броја болесница код којих је урађена хистеректомија класичном (абдоминалном) и минимално инвазивном хирургијом (лапароскопски и вагинални приступ) у Клиници за гинекологију и акушерство Универзитетског клиничког центра Србије.

Методе Укупно 2446 хистеректомија урађено је током периода од пет година. Студија анализира болеснице које су имале хистеректомију у последњих пет година поредећи број и врсту операција пре и током пандемије.

Резултати Укупан пад броја оперисаних болесница био је најизраженији током прве године пандемије. У време пандемије, 2020. и 2021. године постоји стагнација у броју вагиналних и лапароскопских хистеректомија, док се бележи пораст броја абдоминалних хистеректомија. Установљена је високо значајна разлика ($p < 0,01$) између абдоминалних и вагиналних, као и између абдоминалних и ендоскопских хистеректомија.

Закључак Глобални утицај пандемије је због збрињавања симптоматских болесника са ковидом 19 довео до прераспоређивања особља и ресурса, што је значајно утицало на смањење укупног броја операција у многим болницама широм света, а то се највише одразило на елективне, нехитне случајеве.

Кључне речи: ковид 19; гинеколошка хирургија; лапароскопија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Oral intake of bovine lactoferrin alleviates intestinal injury induced by perinatal hypoxia and hypothermia in newborn rats

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SUMMARY

Introduction/Objectives To investigate whether oral administration of lactoferrin attenuates intestinal injury induced by perinatal hypoxia and hypothermia.

Methods Newborn Wistar rat pups were exposed to perinatal asphyxia, followed by global hypothermia. The pups were distributed into two groups: lactoferrin group (LG) – pups that received lactoferrin orally for seven days (20 mg/day), control group (CG) – pups that received normal saline orally during first seven days of life. After seven days macroscopic examination of the bowels and pathohistological analyses of tissue samples have been performed.

Results The incidence of macroscopic injuries was significantly lower in LG group compared to CG. The incidence of pathological findings, as well as the values of injury scores used to assess the intensity and extent of intestinal injury at microscopic level were significantly lower in LG group.

Conclusions Lactoferrin attenuates perinatal hypoxia/hypothermia-induced intestinal injury in newborn rats.

Keywords: lactoferrin; intestinal injury; perinatal hypoxia; hypothermia; neonatology

INTRODUCTION

Close to 80% of all neonatal deaths are due to three leading causes: prematurity and low birth weight, perinatal complications and asphyxia, and infection with variations according to the region and neonatal period [1]. Perinatal asphyxia, especially when combined with uncontrolled accidental hypothermia, may result in decreased perfusion of the gastrointestinal tract, manifesting with vomiting, diarrhea, gastrointestinal hemorrhage and even necrotizing enterocolitis (NEC). The incidence of these events among the neonates born in perinatal asphyxia is around 29% [2].

Intestinal injury caused by hypoxia/reperfusion has been studied in various animal and human models [3–6]. The first histological manifestation of intestinal ischemia is the appearance of small gaps in subepithelial space at the tips of the villi followed by the loss of mature enterocytes. If ischemia lasts long enough, process extends to the base of the villi and causes complete destruction of the villi. Enterocytes located at the top of the villi are most susceptible to injury. Death of enterocytes during hypoxia/reperfusion is a consequence of apoptosis or anoikis (i.e., apoptosis induced by separation of the cells from its natural niches and loss of interplay with the extracellular matrix) [5]. The last mechanism is responsible for the death of enterocytes during hypoxia and apoptosis causes cell death during the reperfusion phase.

Upon termination of hypoxia, in reperfusion phase, the process may go in two directions: migration and proliferation of enterocytes and reestablishment of the continuity of the intestinal epithelium, or amplification of inflammatory response, with neutrophil infiltration and activation of the complement system, what favors apoptosis and leads to further deterioration of the initial epithelial injury and culminates into destruction of all layers of intestinal wall [7].

Hypothermia impairs digestive function and prolongs gastric emptying [8]. At the cellular level, the effects of hypothermia on the gastrointestinal tract are the consequence of redirection of the blood, hemoconcentration, reduction of blood flow and oxygen supply. This initiates a cascade of cellular injury and leads to cell death.

Lactoferrin (LF) is one of the most represented and important bioactive proteins in human and mammal milk. In humans, it is responsible for several actions targeting anti-infective, immunological, and gastrointestinal domains in neonates, infants, and young children. Evidence-based data vouch for the ability of supplemented LF to prevent sepsis and NEC in preterm infants and to reduce the burden of morbidity related to gastrointestinal and respiratory pathogens in young children [9]. LF exists in two forms, as iron (Fe) saturated holo-lactoferrin and as apolactoferrin that do not contain Fe. Two forms of LF differ in their tertiary configurations. LFs of different species differ in secondary and

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tertiary configurations, mostly in the degree glicolization. For potential medical and research purposes in humans, most commonly are used bovine LF, human milk LF and recombinant human LF. LF has diverse biological actions: non-specific defense against bacteria, fungi, and viruses, immunomodulation, modulation of cell proliferation and activation of gene transcription [10]. Action of LF is mediated by several types of LF receptors (LFRs). These receptors modulate inflammation by affecting cell signaling systems, gene expression and transcription of the DNA and post-transcriptional protein processing [11]. The main LFRs are sulfonated proteoglycans that bind up to 80% of the available LF [12]. Especially important LFR, which is present in the enterocytes, is low-density lipoprotein receptor related protein (LDR) whose activation promotes cell mitosis [13]. It is important to note that enterocytes have specific LFR, which is a protein of 34 kDa and which activates the Ras-dependent extracellular signal regulated mitogen activated protein kinase (Ras-ERK) signaling cascade and stimulates proliferation and synthesis of antiapoptotic proteins. Human LF shares ~70% sequence homology with bovine LF [14]. Bovine LF binds to human LFRs and produces all biological effects as LF from human milk [15].

The aim of this experiment was to examine if oral application of LF during the first week of life will reduce incidence and extent of intestinal injury caused by hypoxia in combination with hypothermia.

METHODS

We used adult female Wistar rats and their pups. Females that were in the proestrus phase were mated with sexually mature males (coupled in the same cage for 24 hours and then separated). On the 22nd day of gestation, pregnant females were induced in general anesthesia with ketamine (90 mg/kg) and laparotomy was performed. Blood vessels of the uterus were ligated and the wombs were immediately submersed in a bath tub with 0.9% NaCl heated at 38°C and kept in those conditions for 15 minutes. Pups were delivered by Cesarean section. Immediately after the birth, the pups were reanimated (sweep, aspiration, tactile stimulation). Upon initial reanimation, rapid cooling was conducted in a Styrofoam padded box with adjustable cooling cartridge. Core body temperature of the pups was continuously controlled and measured with rectal probes (RET-3, rectal probe for mice; Physitemp Instruments LLC., Clifton, NJ, USA) and was kept at 32°C. The total duration of hypothermia was one hour, following which the pups were gradually warmed (0.5–1°C/h) in the thermostat, to a normal rectal temperature of 38°C.

The pups were randomly designated in two groups:

1. LF group (LG): 10 pups who survived perinatal hypoxia, exposed to hypothermia, and fed via orogastric tube with 0.2 ml of 10%-suspension (20 mg) of bovine LF (apolactoferrin form of LF; Jarrow Formulas®, Los Angeles, CA, USA) in 0.9% NaCl, once daily. The first dose of LF was given one hour after the birth. Subsequent doses of LF were administered at regular intervals of 24 hours.

2. Control group (CG): 10 pups who survived perinatal hypoxia, exposed to hypothermia and fed via orogastric tube with 0.2 ml of 0.9% NaCl, once daily. The first dose of normal saline was given one hour after the birth and subsequent doses were administered at regular intervals every 24 hours.

After they were adequately labeled, the pups were handed to surrogate mothers. The pups from both groups were breastfed by surrogate mothers, *ad libitum*. The pups were sacrificed on the seventh day of life when laparotomy was made.

Intestines were examined for the presence of any macroscopic changes (discoloration, bleeding, distension or stenosis).

Three tissue samples from the distal part of the ileum (the last 2 cm proximal to the ileocecal valve) were taken from each animal (total of 30 samples in each group). Histological sections were stained with hematoxylin-eosin.

Post-hoc sample size and study power calculation showed that minimum sample size for ideal study power of 80% is 36 histological samples per study group. In our case, 30 specimens were obtained per study group, what sets the study power at the level of 73%. For analysis of macroscopic changes, inclusion of 10 animals per study group, sets study power at 81.7%.

Mucosal injury was assessed in a blinded manner by pathologists. Mucosal injury was quantified using the score previously described by Chiu et al. [3] (0 – indicates absence of mucosal injury (normal finding), values 1–5 indicate different degrees of mucosal injury, 5 being the most devastating).

To access the areal (superficial) distribution of the intestinal injury and compare it between animals, we derived an additional variable – cumulative injury score, defined as the number of positive findings among three histological samples taken from the same animal.

Data are presented as absolute numbers, frequencies, percentages or means ± 2SD. Fisher's exact test, Z-test and Wilcoxon signed-rank test were used for statistical analysis, as appropriate.

The experiment was approved by the Ethical Committee for Animal Care and Use of the University of Novi Sad (approval No 01-237/6).

RESULTS

Macroscopic changes

Discoloration of the intestine was observed in 20% of animals from LG and in 80% of animals from CG. This difference was statistically highly significant (Fisher's exact test; $p = 0.0007$). Other macroscopic changes of the intestines (bleeding, intestinal distension, and stenosis) were not observed in any animal.

Mucosal injury score

Normal histopathological findings were described in 26.67% (8/30) of histological samples in the LG group,

while in the CG were observed in 3.33% (1/30) samples. This difference was statistically significant (Z-test; Z-score is 2.5309; p = 0.0114).

The mean value of mucosal injury score in the LG group was 1.033 ± 1.300. The average value of mucosal injury score in the CG was 2.533 ± 1.363.

The values of mucosal injury scores were significantly lower in group LG (Wilcoxon signed-rank test; p < 0.001), indicating less severe injury in animals from the LG. Distributions of values of mucosal injury scores, in both groups are shown in Figure 1.

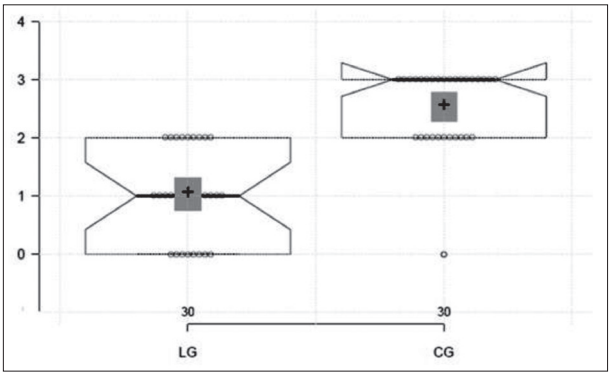

Figure 1. Minimal and maximal values, medians and inter-quartile ranges of mucosal injury scores in lactoferrin group (LG) and control group (CG); center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 95% confidence intervals of the means; data points are plotted as open circles

Cumulative injury score

Normal histopathological findings in all three tissue samples taken from the same animal, were not observed in neither group (there were no animals with cumulative injury score 0).

The mean value of cumulative injury score in the LG group was 1.500 ± 1.054. The average value of cumulative injury score in the CG was 2.900 ± 0.632.

The values of cumulative injury scores were significantly lower in group LG (Wilcoxon signed-rank test; p = 0.005). Distributions of values of cumulative injury scores in both groups are shown in Figure 2.

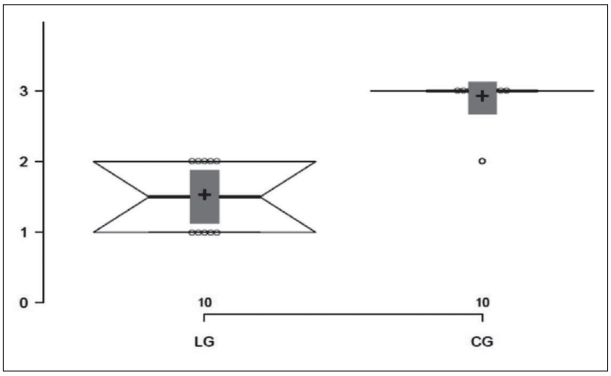

Figure 2. Minimal and maximal values, medians and inter-quartile ranges of cumulative injury scores in lactoferrin group (LG) and control group (CG); center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 95% confidence intervals of the means; data points are plotted as open circles

DISCUSSION

The objective of this study was to investigate whether oral administration of LF attenuate intestinal injury induced by perinatal hypoxia and hypothermia. In this experiment, application of LF was initiated one hour after hypoxia and after process of reperfusion has already begun. LF was administered via orogastric tube, once daily, during a one-week period. It was observed that macroscopic changes of the intestines were more often present in animals that were not fed with LF. The indicators, used to assess the intensity and extent of intestinal injury, showed that intestines were significantly less damaged in animals treated with LF. The animals received 20 mg of LF daily (cca 2000 mg/kg/day). As estimated by the Clark's formula, this dose of LF is equivalent to human dose of 280 mg/kg/day.

In similarly designed experiments conducted by other authors, similar or analogous doses of LF have been applied to experimental animals. For example, Sarkar et al. [16] showed that supplementation with bovine LF in early-life (first seven days, 100 mg/day) with or without added probiotic, reduced the mortality in the suckling piglets by promoting the systemic immunity and enhancing the intestinal integrity.

Cerven et al. [17] studied possible adverse effects of oral administration of LF (1000 mg/kg/daily), during a four-week period. Their results showed no toxic or adverse effects on nutritional, hematological or biochemical status of experimental animals.

In a recently published review article by Ashraf et al. [18] the authors stated that the European Food Safety Authority approved bovine LF as a safe novel food additive for infant formula at the level of 1000 mg/l, and recommend that the highest intake of bovine LF for infants should be 1.1 g/day. Referring to different literal sources, the same group of authors suggested that LF produce desired positive effects when it is added to infant formula at the level of 850 mg/l or 600 mg/kg/day (what is twofold higher dose than estimated human dose used in our study) [18].

In serval in vitro experiments, it was found that, in dose-dependent manner, LF stimulates proliferation and differentiation of intestinal epithelium by interfering with the processes of DNA transcription, promotes production of transformational growth factor-β1 and secretion of interleukin-18. The 2017 Cochrane review included six randomized controlled trials, and meta-analysis suggested that substantial reductions in late-onset infection and NEC was associated with LF supplementation in very preterm infants [19]. Other experiments showed that LF reduces production of free radicals and lipid peroxidation products and thereby minimizes the effects of inflammation on intestinal mucosa [20]. Experiments on adult rats, conducted

by Zhang et al. [20], demonstrated that orally administered LF had protective effect on intestinal ischemia-reperfusion injury if LF was applied prior induction of hypoxia (as premedication, during preparation for elective surgery of the intestine). This protective effect was dose-dependent and was attributed to inhibition of pro-inflammatory cytokines, inhibition of apoptosis and increased local production of tissue antioxidants.

The exact mechanism through which LF exerts protective effect on enterocytes has not been fully elucidated. In the intestine LF is partially degraded to polypeptide fragments that bind to receptors on the enterocytes, lymphocytes and dendritic cells, and trigger intracellular signaling cascades, stimulate proliferation and suppress proapoptotic processes, thus maintaining the integrity of the intestinal barrier. LF can be transported into the cytoplasm or the nucleus of cells, where it can bind to DNA and alter expression of genes, including those that control apoptosis and cell death [21]. LF modulate expression of more than twenty genes associated with immune response, suppresses the production of tumor necrosis factor alpha (TNF-α) and Interleukins IL-1β, IL-6 and IL-8 in human mononuclear cells and improves production of IL-10 and IL-4 [22]. LF stimulates increased intestinal stem cell marker Lgr5 + expression and increased nuclear β-catenin - indicating upregulated Wnt pathway, as well as increased Ki67 positivity, suggesting enhanced proliferation [23]. By binding to micro-organisms or their toxic products, LF directly regulates composition of intestinal microbiome [12].

In our experiment, we used iron-free form of bovine LF (apolactoferrin). The question arises whether exogenous LF may interact with the LFRs on human enterocytes and cause any biological effect? Jiang and al. demonstrated that both forms of human LFs bind to receptors on human enterocytes and subsequently may be transported within enterocytes, allowing involvement in intracellular processes and signaling [24]. However, these authors showed that effects of apo- and holo-lactoferrin were different and that only apolactoferrin stimulated proliferation of enterocytes. This difference is explained by the different tertiary configuration of these two types of LF (holo-lactoferrin is less reactive) and the higher affinity of cellular receptors for apolactoferrin.

The results of our experiment indicate that repeated, oral administration of LF attenuates intestinal injury induced by hypoxia. On the other hand, data reported by the ELFIN trial investigators group, showed that enteral LF supplementation (150 mg/kg per day until 34 weeks' postmenstrual age) does not reduce the risk of late-onset infection, other morbidity, or mortality in very preterm infants [25].

CONCLUSION

Our experiment showed that repeated, oral administration of bovine LF attenuates intestinal injury induced by perinatal hypoxia/hypothermia in newborn rats. Similar data, from other animal and human-based studies have been reported in literature. Most experiments and studies demonstrated positive effects of bovine LF with no toxic or adverse effects, so we may assume that bovine LF is a potent agent that may be safely used for prevention of post-hypoxic intestinal injury in neonates. The precisely assessed dosage, methods of application and other details of LF application are still controversial and additional studies on this topic are needed.

Conflict of interest: None declared.

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Орална примена говеђег лактоферина делује протективно на оштећење црева индуковано перипарталном хипоксијом и хипотермијом код новорођених пацова

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САЖЕТАК

Увод/Циљ Циљ рада је био истражити да ли орална примена лактоферина ублажава интестинално оштећење изазвано перипарталном хипоксијом и хипотермијом.

Методе Младунци пацова соја вистар су одмах по рођењу изложени хипоксији/асфиксији и глобалној хипотермији. Распоређени су у две групе: лактоферин група – младунци који су примали лактоферин орално током седам дана (20 *mg*/дан) и контролна група – младунци који су примали физиолошки раствор орално током првих седам дана живота. После седам дана урађени су макроскопски преглед црева и патохистолошка анализа узорака ткива.

Резултати Инциденција макроскопских оштећења била је значајно нижа у групи младунаца који су примали лактоферин у односу на контролну групу. Инциденција патолошких налаза, као и вредности хистолошких скорова који су коришћени за процену интензитета и обима оштећења црева биле су статистички значајно ниже у групи која је третирана лактоферином.

Закључак Лактоферин ублажава интестиналну повреду изазвану перипарталном хипоксијом/хипотермијом код новорођених пацова.

Кључне речи: лактоферин; оштећење црева; перипартална хипоксија; хипотермија; неонатологија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Analysis of patients with adhesive capsulitis treated at the Kosovska Mitrovica Clinical Hospital Center over a two-year period

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SUMMARY
Introduction/Objective Adhesive capsulitis (AC) is a condition characterized by pain and significant reduction in active and passive movements in the glenohumeral joint, especially in external rotation. It is one of the most common and challenging clinical disorders encountered by orthopedic surgeons. AC is predominantly an idiopathic condition and has an increased prevalence in women and patients with diabetes mellitus and hypothyroidism. The etiology and pathogenesis are not entirely clear. Treatment options include conservative and surgical approaches, but the results remain controversial.
Methods The study included patients treated for primary (idiopathic) AC from June 2021 to June 2023 at the Kosovska Mitrovica Clinical Hospital Center. A total of 172 patients were treated. All were managed non-operatively with physical therapy and local intra-articular steroid injections. The patients were followed up on an outpatient basis monthly, then at six months, one year, and two years.
Results All patients were divided into two groups. The first group (87 patients) underwent physical procedures, while the second group (85 patients) received intra-articular corticosteroid injections. Patients that were treated with physical therapy were not administered injections of steroids, while the group of patients treated with corticosteroids were not given physical therapy. Patients were selected through randomization.
Conclusion Steroid injections may be beneficial in the early stages of the disease, especially in the first 6-8 weeks, but long-term results did not show any significant difference between the two groups of patients.
Keywords: adhesive capsulitis; shoulder; physical therapy; steroid therapy

INTRODUCTION

Primary adhesive capsulitis (AC) of the shoulder, or "frozen shoulder," is an aseptic inflammation of the joint capsule. It was first described in the late 19th century as a condition that is "difficult to define, difficult to treat, and difficult to explain" [1, 2, 3]. AC is a common cause of shoulder pain, restricted mobility, and disability [4]. The characteristic feature of this disease is spontaneous chronic pain with a gradual and progressive loss of both active and passive movements in the shoulder joint [2]. The prevalence of AC ranges 2–5% of the general population, although estimates vary from 0.5% to as high as 10% [3, 4]. It is about three times more common in the female population, usually occurring between the fourth and the seventh decade of life, but patients' ages can range 27–85 years [5]. It can be primary (idiopathic) or secondary. Primary idiopathic frozen shoulder (FS) is often of unknown cause but is frequently associated with other diseases and conditions, such as diabetes mellitus, and can be the first presentation in diabetics. According to some data, up to 20% of patients with diabetes mellitus develop AC [6, 7, 8]. Secondary AC can occur after shoulder injuries or immobilization (e.g. rotator cuff tendon tear, biceps tenosynovitis and calcific tendinitis, hemiparesis) [9, 10]

Despite its prevalence, FS is one of the least understood shoulder conditions. Its definition, classification, pathophysiology, diagnosis, natural course, treatment, and prognosis remain controversial [9]. Optimal treatment for AC has been the subject of significant debates, especially because the condition tends to resolve spontaneously months after the onset of symptoms [6]. The primary goal of the study was to compare the therapeutic effectiveness of local corticosteroid application and physical therapy in the treatment of AC.

METHODS

A prospective study included 172 patients who were randomly divided into two groups through randomization. Randomization was

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performed after obtaining written consent from study participants. Each patient completed a questionnaire that included the Oxford Shoulder Score. The patients were followed up on an outpatient basis over a two-year period from June 2021 to June 2023. The study extracted the following data: demographics (sex, age), duration of symptoms before treatment, comorbidities (diabetes mellitus, hypothyroidism), interventions (location and number of injections, doses and volume of corticosteroids, types of corticosteroids), assessment periods, and outcomes. Patients under 18 years of age, those with contraindications for corticosteroid therapy, patients with secondary AC (inflammatory, degenerative, metabolic), individuals in whom AC developed as a result of shoulder fractures, were not included in the research.

For all patients, a detailed medical history was taken upon admission, a complete clinical examination of the shoulder was performed, routine radiography and ultrasound examination were conducted. Inclusion criteria for the study were pain and limited range of motion. Symptoms were required to be present for at least five months before the start of the study. Shoulder pain should have been present at least 50% of the time during the day, with pain occurring on acute shoulder elevation, night pain, and pain during passive movements in at least two directions (abduction, flexion, external and internal rotation). Passive movements in the shoulder should have been reduced by more than 30%.

The following physical procedures were used: transcutaneous electrical nerve stimulation, mobilization techniques, active range of motion exercises, and cryotherapy (application of ice).

We first anesthetized the skin of patients receiving corticosteroids with 2% lidocaine. Then, using ultrasound guidance, with 21-gauge needles, 2½–3" in length, we applied previously prepared solutions of betamethasone (7 mg/ml) into the intra-articular space of the shoulder joint. The injections were administered in outpatient conditions with the complete application of all antiseptic methods. The patient was positioned on their back with the arm by their side and in a sitting position with the arm straight in a neutral position. The first injection was given at the beginning of the treatment, the second after a month and a half, and the third three months after the first examination. We continued the follow-up of the patients on an outpatient basis in the sixth month, then in the first and second years.

We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (Ethics Committee Decision No. 1814/11). Written consent to publish all shown material was obtained from the patients.

RESULTS

Depending on the type of variables and the normality of their distribution, data description is presented as n (%), mean ± sd, or median (min–max). Statistical hypothesis testing methods used included t-test, Mann–Whitney test, χ^2 test, and Fisher's exact test. Logistic regression with mixed effects was employed for modeling the relationships between dependent variables in repeated measurements and potential predictors. In multivariate regression models, predictors from univariate analyses that were statistically significant at a significance level of 0.05 were included. Statistical hypotheses were tested at a significance level (alpha level) of 0.05. All data were processed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) or the R programming environment (R Core Team, 2018).

During the study, we analyzed demographic data (sex and age). There were a total of 113 female participants (65.7%), while there were 59 males (34.3%). The youngest patient was 38 years old, and the oldest one was 69 years old, with a mean age of 53.76 years (Table 1).

The time from the onset of the first symptoms to the first examination lasted from five to 12 months with an average value of around eight months (Table 2).

The majority of patients with AC had diabetes mellitus (35 of them, 20.3%), 16 patients (9.3%) had issues with reduced thyroid function along with AC, while a total of 100 patients (58.1%) had no accompanying diseases. Glycated hemoglobin (HbA1c) was determined for all patients. If HbA1c was above 6.5%, the patient was classified as having diabetes mellitus, following international guidelines (Table 3).

Table 1. Sex distribution of patients

Sex		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	female	113	65.7	65.7	65.7
	male	59	34.3	34.3	100
	total	172	100	100	/

Table 2. Statistical indicator of pain-free period in adhesive capsulitis

χ^2 tests						
/	Value	df	Asymptotic significance (2-sided)	Exact sig. (2-sided)	Exact sig. (1-sided)	Point probability
Pearson χ^2	0.003[a]	1	0.960	1.000	0.544	/
Continuity correction	0.000	1	1.000	/	/	/
Likelihood ratio	0.003	1	0.960	1.000	0.544	/
Fisher's exact test	/		/	1.000	0.544	/
Linear-by-linear association	0.003[c]	1	0.960	1.000	0.544	0.127
N of valid cases	172	/	/	/	/	/

[a]0 cells (0%) have expected count less than 5; the minimum expected count is 29.16;
[b]computed only for a 2 × 2 table;
[c]standardized statistic is 0.050

Table 3. The relationship of comorbidities with adhesive capsulitis

Comorbidities		Group		Total
		Corticosteroids	Physical therapy	
Diabetes mellitus	count	18	17	35
	% within group	21.2%	19.5%	20.3%
Hyperthyroidism	count	6	10	16
	% within group	7.1%	11.5%	9.3%
Rest	count	11	10	21
	% within group	12.9%	11.5%	12.2%
No comorbidities	count	50	50	100
	% within group	58.8%	57.5%	58.1%
Total	count	85	87	172
	% within group	100%	100%	100%

Table 4. Statistical indicator of limited mobility in adhesive capsulitis

χ^2 tests						
/	Value	df	Asymptotic significance (2-sided)	Exact sig. (2-sided)	Exact sig. (1-sided)	Point probability
Pearson χ^2	1.053[a]	3	0.788	0.803	/	/
Likelihood ratio	1.064	3	0.786	0.803	/	/
Fisher's exact test	1.071		/	0.807	/	/
Linear-by-linear association	0.018[b]	1	0.893	0.901	0.471	0.049
N of valid cases	172	/	/	/	/	/

[a]0 cells (0%) have expected count less than 5; the minimum expected count is 7.91;
[b]standardized statistic is -0.135

Table 5. The relationship between age and type of therapy

Case summaries						
Group	N	Mean	Std. deviation	Median	Minimum	Maximum
Corticosteroids	85	54.14	6.842	54	38	69
Physical therapy	87	53.38	7.071	54	37	68
Total	172	53.76	6.949	54	37	69

All patients who participated in the study had shoulder pain, often pain that was present even at night during the resting phase. Restricted movement, primarily in terms of external rotation, was present in 141 patients (82%), followed by abduction in 82 patients, or 47.7%, internal rotation in 47 patients (27.3%), and flexion in 53 patients (30.8%) (Table 4).

The total number of patients receiving corticosteroids was 85 (49.4%). Physical therapy was applied to 87 patients (50.6%) (Table 5).

During the observed period, there was an increase in the frequency of pain improvement ($b = 1.423$; $p < 0.001$), especially in the corticosteroid group ($b = 9.515$; $p < 0.001$). Overall, during the observed period, there was a significant increase in the frequency of movement improvement ($b = 1.736$; $p < 0.001$), with the corticosteroid group having a significantly higher frequency of pain improvement ($b = 3.545$; $p = 0.001$).

In the multivariate mixed-effect regression model with movement improvement as the dependent variable, variables that were statistically significant in univariate models at the significance level of 0.05 were included. Statistically significant predictors of movement improvement were the use of corticosteroids compared to physical therapy ($B = 4.232$; $p = 0.009$) and younger participants ($B = -0.270$; $p = 0.031$) (Table 6).

In the multivariate mixed-effect regression model with pain improvement as the dependent variable, variables that were statistically significant in univariate models at the significance level of 0.05 were included. Statistically significant predictors of pain improvement were the use of corticosteroids compared to physical therapy ($B = 8.481$; $p < 0.001$) and the absence of internal rotation ($B = -2.917$; $p = 0.022$) (Table 7).

DISCUSSION

Despite its prevalence, FS is one of the least understood shoulder conditions. It is a condition frequently encountered by rheumatologists, rehabilitation professionals, and orthopedic surgeons. The term "frozen shoulder" is often used loosely and mistakenly attributed to other shoulder limitations, such as rotator cuff tears or osteoarthritis. Subacromial pathology (e.g. rotator cuff tendinopathy, subacromial bursitis, and impingement syndrome) can also closely resemble AC in its early stages [4]. AC is known to be a benign and self-limiting disease that spontaneously resolves in about two years, but some patients remain symptomatic, with significant movement restrictions in the shoulder, even years after the onset of the disease [11, 12]. Only 59% of patients regain normal function after four years [13]. The American Shoulder and Elbow Surgeons define AC as a "condition of uncertain etiology characterized by significant restriction of both active and passive shoulder motion in the absence of a known intrinsic shoulder disorder" [3]. AC is characterized by pain, typically worsening at night, a poorly localized dull ache that can radiate to the biceps [14]. Impaired range of motion with forward flexion, abduction, external rotation, and internal rotation is a cardinal clinical finding for AC. In advanced disease, observing the patient's gait may reveal a loss of the natural swing of the arm during walking.

Diagnosis, pathophysiology, course, treatment, and prognosis remain unclear [9]. Diagnosis is primarily clinical, based on a well-taken history and a comprehensive clinical examination, requiring a comparative examination of both shoulders [15]. Ultrasonography has shown high accuracy for diagnosing AC of the shoulder. Therefore, it has the potential to be adopted as a desirable modality in diagnosing FS [16]. The method is fast, inexpensive, and offers dynamic possibilities for examining the subject in multiple planes. The sonographic parameters studied include the thickness of the coracohumeral ligament, increased soft tissue in the rotator interval (static parameters), and restriction of abduction and external rotation

Table 6. Mixed-effect regression models with movement improvement as the dependent variable

Variables	Univariant		Multivariant	
	B	P	B	p
Groups (Corticosteroids/PT)	3.545	0.001	4.232	0.009
Sex (female/male)	-1.127	0.386	/	/
Age	-0.211	0.041	-0.270	0.031
Time, symptoms, first diagnosis	-0.421	0.291	/	/
Comorbidities				
Without	referent category		/	
Diabetes mellitus	-1.586	0.328	/	/
Hyperthyroidism	-1.830	0.397	/	/
Rest	0.436	0.821	/	/
Pain before treatment	21.183	0.995	/	/
Limited movement	-20.465	0.997	/	/
Outer rotation	-0.836	0.609	/	/
Inner rotation	1.595	0.251	/	/
Abduction	-2.581	0.051	/	/
Flexion	-0.981	0.461	/	/

PT – physical therapy

Table 7. Mixed-effect regression models with pain reduction as the dependent variable

Variables	Univariant		Multivariant	
	B	P	B	p
Groups (Corticosteroids/PT)	9.515	< 0.001	8.481	< 0.001
Gender (female/male)	-1.035	0.361	/	/
Age	-0.044	0.573	/	/
Time. symptoms. first diagnosis	0.187	0.588	/	/
Comorbidities/				
Without	referent category			
Diabetes mellitus	-1.328	0.337	/	/
Hyperthyroidism	-0.567	0.762	/	/
Rest	0.043	0.980	/	/
Pain before treatment	2.428	0.653	/	/
Limited movement	-	-	/	/
Outer rotation	1.795	0.196	/	/
Inner rotation	-3.103	0.021	-2.917	0.022
Abduction	0.289	0.789	/	/
Flexion	-2.730	0.036	-2.193	0.055

PT – physical therapy

during dynamic scanning. AC diagnosis is clinical, and the use of MRI should be reserved for evaluating other sources of shoulder pathology rather than confirming AC diagnosis [11, 14].

The pathophysiology of idiopathic AC is poorly understood [6]. For this reason, the treatment of this disease remains controversial. Thus, determining the biological pathophysiology of FS is a key milestone in developing new treatments for patients with FS [12]. The pathophysiology of this disease supports the theory that AC results from a complex chain of events starting with inflammation leading to fibrosis and contraction of the shoulder capsule, the so-called "inflammatory-fibrinous cascade" [3, 8, 14]. Collagen fibers adhere to the glenohumeral ligaments, tendons, and joint surfaces, causing contracture and stiffness of the joint. Therefore, even after inflammation subsides, adhesions persist, significantly restricting movements in the shoulder [10]. It remains to be discovered what triggers this cascade and leads to the acute onset of AC. Changes also occur in the surrounding periarticular tissue involving ligaments, tendons, and muscles. As a result, we get the FS. Patients cannot lift their arm above the shoulder, throw a ball, perform a movement behind the back, make a quick movement in the shoulder, and cannot sleep on the painful side. The shoulder joint capsule in AC contracts and significantly thickens, resulting in pain and stiffness of the shoulder capsule, leading to a reduction in the range of motion in the shoulder. Women with diabetes have a 25% chance of developing AC at least once in their lifetime. Patients with diabetes are often described as having a worsened disease course [5, 7, 10], explained by the theory that diabetes mellitus is a chronically inflammatory condition [11, 17], with an excessive concentration of glucose causing increased cross-linking of collagen and stabilization of connective tissue [12].

Treatment goals include pain relief, restoring movement, and regaining shoulder function [3]. Any treatment that reduces the duration of the disease to less than 24 months is considered a success in treatment, reflecting the severity and complexity of treating FS, which hinders normal daily activities for an extended period. Initial conservative treatment can be successful in up to 90% of patients. Most cases of AC can be treated within primary health care. Clinicians are encouraged to initiate treatment with patient education. In the initial inflammatory phase, it is crucial not to trigger a more significant inflammatory response by treatment procedures. The primary focus is to control pain and inflammation with analgesics and anti-inflammatory medications. In the second phase, when pain subsides, treatment includes ultrasound, pulsed magnetic field therapy, shockwave therapy, transcutaneous electrical nerve stimulation, laser, and interferential currents. Intensive stretching should still be avoided at this stage. Only in the third phase of the disease, an intensive physiotherapy protocol is implemented to achieve the maximum range of motion, strengthen muscles, and restore shoulder joint function [4, 12, 14].

When patients experience the most pain, steroid injections can be beneficial in the early stages of the disease (especially in the first six weeks). However, long-term results show no significant difference between patients treated with steroids and control groups treated with other nonoperative methods. Most studies used only a single corticosteroid injection, while two studies used multiple corticosteroid injections [5, 7]. Intra-articular corticosteroid is widely used as a conservative treatment for AC due to its cost-effectiveness and patient acceptance [8, 12]. As AC is assumed to be an inflammatory and fibrotic disease, early treatment with intra-articular corticosteroid injections may reduce synovitis, limit the development of capsular fibrosis, and alter the natural history of the disease [5, 8, 9]. Our results confirm those reported by Van der Windt et al. [7], showing that the beneficial effects of corticosteroid injection are superior to supervised physiotherapy programs.

Based on the pathophysiology of AC, it could be assumed that corticosteroid injections would be most

effective in the earlier inflammatory stages of the disease, rather than in the later stages when fibrotic contracture is more evident. In a study by Kim et al. [17], a significant improvement in pain outcomes was observed after four weeks in diabetic patients receiving intra-articular steroid injections compared to those who did not receive any injection ($p = 0.020$).

CONCLUSION

The AC commonly encountered in general orthopedic practice is a condition of pain and stiffness with resultant functional impairment. Appropriate decisions regarding the treatment of AC require a comprehensive understanding of pathophysiology, the patient's overall health condition, functional requirements, symptom severity, and response to non-operative treatment. Most patients will experience complete resolution with conservative treatment; therefore, conservative therapy should be the first option. Given the recent increase in risk factors for AC, such as diabetes mellitus, research is needed to investigate whether the incidence of AC has also increased.

The results of this study show that in patients with AC a single intra-articular injection of corticosteroids applied under ultrasound guidance, along with a simple home exercise program, is superior to a physiotherapy program in improving shoulder pain and function after six weeks. Steroid injections may be beneficial in the early stage of the disease (especially within the first six weeks). Other important questions that remain to be clarified include whether the accuracy of needle placement, anatomical location, frequency, dose, and the type of corticosteroid affect effectiveness. With our study, we provide compelling evidence that intra-articular)corticosteroid is associated with better short-term outcomes than other treatments, with potential benefits extending into the medium term; therefore, we recommend their early use with a concurrent home exercise program. This can be complemented with physiotherapy to further increase the chances of symptom resolution within six months.

Conflict of interest: None declared.

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Анализа болесника са адхезивним капсулитисом лечених у Клиничко-болничком центру „Косовска Митровица“ у двогодишњем периоду

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САЖЕТАК

Увод/Циљ Адхезивни капсулитис је болест која се карактерише болом и значајним ограничењем активних и пасивних покрета у гленохумералном зглобу, нарочито у спољној ротацији. То је један од најчешћих, али и веома изазовних клиничких поремећаја са којима се сусрећу ортопедски хирурзи. Адхезивни капсулитис је претежно идиопатско стање и има повећану преваленцију код жена и код болесника са дијабетесом мелитусом и хипотиреозом. Етиологија и патогенеза су недовољно јасне. Лечење је конзервативно и хируршко, али су резултати и даље контроверзни.

Методе У рад су укључени болесници лечени од примарног (идиопатског) адхезивног капсулитиса у периоду од јуна 2021. до јуна 2023. године у Клиничко-болничком центру „Косовска Митровица“. Укупно су лечена 172 болесника. Сви су лечени неоперативно, физикалном терапијом и локално – инјекцијом стероида, интраартикуларно. Болесницима који су лечени физикалном терапијом није апликована инјекција стероида, док групи болесника који су лечени кортикостероидима нисмо укључивали физикалне процедуре. Болесници су праћени амбулантно једном месечно, потом на шест месеци, годину дана и две године.

Резултати Све болеснике смо поделили у две групе. Прва група (87) болесника подвргнута је физикалним процедурама, док су другој групи (85) апликоване интраартикуларне инјекције кортикостероида. Болесници су бирани рандомизацијом.

Закључак Инјекције стероида могу бити од користи у раном периоду болести, нарочито у првих шест до осам недеља, али дугорочни резултати нису показали никакву разлику између две групе болесника.

Кључне речи: адхезивни капсулитис; раме; физикална терапија; стероидна терапија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Ultrasound and laboratory parameters in distinguishing complicated from uncomplicated appendicitis in children

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SUMMARY

Introduction/Objective The objective was to evaluate sonographic and laboratory findings as predictors of complicated and uncomplicated appendicitis in order to decide on further treatment options.

Methods This is a retrospective cohort study of 174 pediatric patients who had laboratory tests and ultrasound done before appendectomy during a one-year period. Results were compared with the intraoperative and histopathological findings of complicated (gangrenous or perforated) or uncomplicated (phlegmonous) appendicitis and assessed by binary logistic regression with backward elimination. The initial model included eight predictors. After backward elimination four remained: periappendiceal fluid, hyperechoic periappendiceal fat, white blood cell (WBC) count and C-reactive protein (CRP). The final model included the interaction between periappendiceal fluid and hyperechoic periappendiceal fat. Diagnostic performance of each parameter was presented with sensitivity and specificity.

Results Out of all patients, 86 had uncomplicated and 88 had complicated appendicitis (37 gangrenous, and 51 perforated). In the final model three predictors were significantly associated with complicated appendicitis: interaction between periappendiceal fluid and hyperechoic periappendiceal fat, WBC count > 11 × 10^9/l, and CRP > 100 mg/l. Inclusion of interaction between periappendiceal fluid and hyperechoic periappendiceal fat excluded them as individual predictors. The maximum outside appendiceal diameter of more than 6 mm had the highest sensitivity (93.2%), while wall thickness > 3 mm was the most specific (95.2%).

Conclusion Using periappendiceal fluid and hyperechoic periappendiceal fat as sonographic predictors and WBC and CRP as laboratory predictors can differentiate uncomplicated from complicated appendicitis in children and help a physician decide on antibiotic or surgical treatment.

Keywords: ultrasound; laboratory parameters; complicated appendicitis; uncomplicated appendicitis; children

INTRODUCTION

Appendicitis is the most common cause for emergency surgery in children. Certain laboratory parameters [white blood cell (WBC) count, C-reactive protein (CRP), total neutrophil count and procalcitonin] have predictive value, but they are considered nonspecific [1, 2]. Many other nonsurgical and surgical entities such as mesenteric adenitis, Crohn's disease, infectious enterocolitis, epiploic appendagitis, omental infarction, intussusception, ovarian torsion, and urolithiasis can cause pain in the right iliac fossa. Therefore, combining clinical, laboratory, and imaging findings remains essential for the definitive diagnosis [3].

The interest in the non-operative management of appendicitis has grown most likely due to a growing number of randomized studies showing postoperative complications and higher operative treatment costs. Additionally, when using antibiotics as first-line therapy, appendectomy can be avoided in significant number of patients [4–8]. This non-surgical approach is reserved for patients with uncomplicated appendicitis, without signs of gangrene or perforation, while complicated appendicitis treated this way leads to higher risk of surgical complications and subsequent surgery [6, 9].

Because of its noninvasive nature, availability, high diagnostic accuracy, lack of radiation and contrast administration, ultrasonography is the diagnostic modality of choice in pediatric patients [10]. Studies have shown that it is a reliable imaging method for the differentiation of perforated and non-perforated appendicitis when relying on highly specific findings such as periappendiceal fluid and the loss of the conspicuity of the echogenic submucosal layer [11, 12, 13].

The aim of our study was to evaluate sonographic and laboratory findings as predictors of complicated and uncomplicated appendicitis in order to decide on further treatment options.

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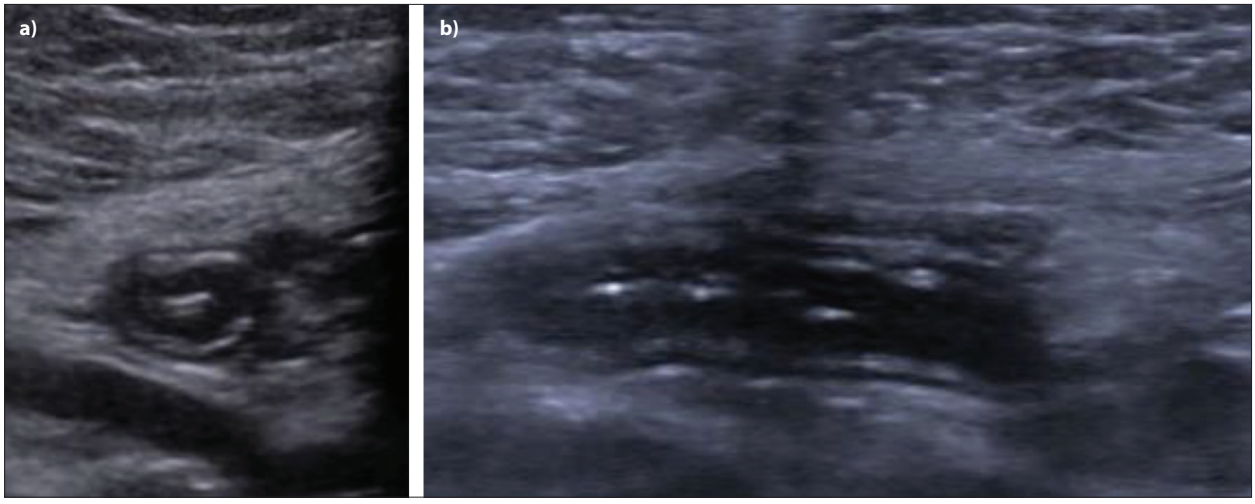

Figure 1. Acute uncomplicated appendicitis in a seven-year-old boy with a one-day history of abdominal pain, vomiting, and diarrhea; axial (a) and longitudinal (b) grayscale ultrasonography images of the right lower quadrant shows noncompressible 8 mm appendix with wall thickening and typical target sign

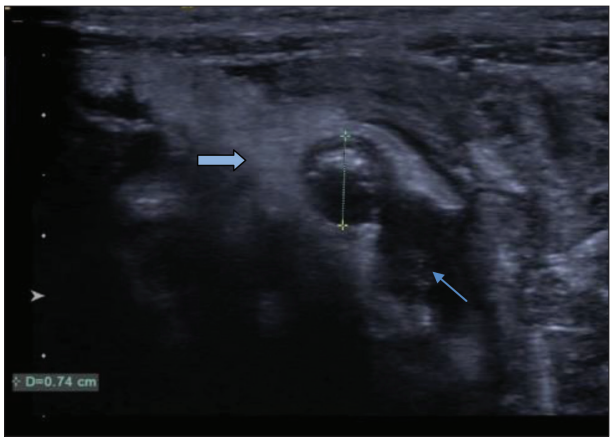

Figure 2. Grayscale axial ultrasonography images of the right lower quadrant in a five-year-old girl with a one-day history of abdominal pain shows a 7.4 mm in diameter noncompressible appendix with hyperechoic periappendiceal fat (bold arrow), wall thickening, and periappendiceal free fluid (thin arrow); complicated (perforated) appendicitis was found at appendectomy

METHODS

This is a retrospective cohort study done at a Tertiary Pediatric Institution approved by Institutional Ethics Committee. This study included 174 patients aged 2–18 years who had laboratory tests and ultrasound done by a pediatric radiologist before appendectomy between January 2022 and January 2023.

Patient population

We used our hospital's information system to review medical charts of all patients who received an ultrasound examination before the operation, had appendectomy during the same hospital admission as sonography, and had intraoperatively or histopathologically proven appendicitis. Patients who had some data missing due to incomplete data entry or had some alternative diagnosis proven (carcinoid), were excluded from the study, so the final number of the patients was 174.

Diagnostic protocol

Ultrasonography examinations were performed by one of the attending radiologists from our department on Siemens Acuson s2000 (Siemens Medical Solutions USA, Inc., Malvern, PA, USA), using convex and linear transducers (2–6 mHz and 4–9 mHz). The whole abdomen was scanned, with special interest for the right lower quadrant. The grayscale images in long and short axis were made as well as color Doppler images.

According to the previous studies we reviewed the following sonographic findings in each patient: the maximum outside diameter, wall thickness, periappendiceal fluid, periappendiceal hyperechoic fat, lymphadenitis and appendicoliths [12, 13, 14]. The maximum outside diameter was measured in short axis view and it was considered significant when it was 6 mm or more (Figure 1). Wall thickness was considered significant when measured over 3 mm. Periappendiceal fluid was diagnosed in direct proximity of the appendix, while periappendiceal hyperechoic fat was defined as increased echogenicity of the tissue adjacent to the appendix (Figure 2). Lymphadenitis was defined as sonographically detectable lymph nodes. An appendicolith was diagnosed when we identified an intraluminal hyperechogenic focus with an acoustic shadow (Figure 2). Because of the different therapeutic approach for patients with appendiceal abscess or inflammatory mass, they were excluded from this study [15]. Laboratory findings that were used as predictors were white blood cell (WBC) count over 11×10^9/l and C-reactive protein (CRP) over 100 mg/l [16].

Intraoperative and histopathological findings

Based on the intraoperative findings and histopathological findings, appendicitis was classified into three groups: phlegmonous, gangrenous, and perforated. Phlegmonous appendicitis was defined by transmural neutrophil infiltration without gangrene and perforation, gangrenous appendicitis was characterized by foci of ischemia that cause

Table 1. Distribution of age, sex, sonographic and laboratory findings

Parameters	Total (n = 174)	AUA (n = 86)	ACA (n = 88)
Age	12 (2.5–18)	9.3 (2.5–18)	8.9 (4–18)
Male	108 (62%)	54	54
Female	66 (38%)	35	31
Maximum outside diameter (mm)	9.24 ± 2.53	9.51 ± 2.58	8.97 ± 2.54
Wall thickness > 3 mm	14 (8%)	6 (7%)	8 (9%)
Periappendiceal fluid	73	20	53
Periappendiceal hyperechoic fat	117	49	68
Lymphadenitis	76	43	33
Appendicoliths	38	17	21
White blood cell count	15.5 ± 5.26	14.54 ± 5.22	18.16 ± 5.38
C-reactive protein level, med (min–max)	41.2 (0.3–225)	23.30 (0.3–225)	60.90 (2.2–225)

AUA – acute uncomplicated appendicitis; ACA – acute complicated appendicitis

gangrene. Perforation was determined by the presence of a transmural defect. Phlegmonous appendicitis was considered to be uncomplicated while gangrenous and perforated were designated as complicated [17].

Statistical analysis

All statistical analyses were calculated using the Statistical Package for Social Sciences IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). Sonographic findings and laboratory parameters were compared with intraoperative and histopathological findings of complicated and uncomplicated appendicitis and assessed by binary logistic regression. A backward variable elimination was performed to determine a proper model for the regression analysis. The initial model included eight predictors: the maximum outside appendiceal diameter, wall thickness, periappendiceal fluid, hyperechoic periappendiceal fat, lymphadenitis, presence of appendicoliths, WBC count and CRP level. After backward elimination only four remained: periappendiceal fluid, hyperechoic periappendiceal fat, WBC count and CRP level. In the final step, third model was constructed by including the interaction between periappendiceal fluid and hyperechoic periappendiceal fat. In the final model there were three significant predictors: WBC count, CRP, and interaction between periappendiceal fluid and hyperechoic periappendiceal fat. The threshold for assessing statistical significance was set to 0.05. Diagnostic performance of each parameter was presented with sensitivity and specificity.

RESULTS

There were 174 patients who met the inclusion criteria, 108 male (62%) and 66 female (38%). The age range was from two years and six months to 18 years, with a mean age of 12 years. Out of all patients, 86 (49.43%) had histopathologically proven uncomplicated appendicitis, and 88 (50.57%) had complicated appendicitis (37 gangrenous (21.3%) and 51 perforated (28.73%)). Mean maximum outside diameter of appendix was 9.24 mm. Mean age of patients with complicated appendicitis was 8.9 years and was significantly younger than the mean age of patients with uncomplicated appendicitis which was 10.3 years ($p < 0.005$) (Table 1).

After the binary logistic regression was performed, the following predictors showed significant correlation with complicated appendicitis: periappendiceal fluid had odds ratio (OR) of 4.93 with $p < 0.001$, hyperechoic periappendiceal fat (OR = 2.17, $p = 0.047$), WBC count > 11×10^9/l (OR = 3.58, $p = 0.028$), CRP > 100 mg/l (OR = 3.72, $p = 0.003$). In the final model, we included interaction between periappendiceal fluid and hyperechoic periappendiceal fat which showed OR of 8.63 and $p < 0.001$. Inclusion of interaction between these two variables excluded them as individual predictors (Table 2).

Table 2. Binary logistic regression with backward elimination

Finding	OR	p value
Periappendiceal fluid	4.93	< 0.001
Hyperechoic periappendiceal fat	2.17	0.047
WBC count > 11×10^9/l	3.58	0.028
CRP > 100 mg/l	3.72	0.003
Interaction between periappendiceal fluid and hyperechoic periappendiceal fat	8.63	< 0.001

OR – odds ratio; WBC – white blood cells; CRP – C-reactive protein

The maximum outside appendiceal diameter of more than 6 mm was the most sensitive parameter of complicated appendicitis (93.2%), but it had a very low specificity (8.6%). When the diameter threshold was increased (over 6mm), the specificity values were higher, but had a concurrent decrease of sensitivity values. The most specific (95.2%) sonographic finding for the complicated appendicitis was wall thickness > 3 mm, with a lower sensitivity (16.6%) (Table 3).

DISCUSSION

Some studies have indicated antibiotic-only treatment for patients with uncomplicated appendicitis [5–9]. This has made it necessary to establish clinical, laboratory, and imaging findings that would accurately distinguish it from complicated appendicitis and ensure the complications

Table 3. Sensitivity and specificity values of sonographic and laboratory findings for acute complicated appendicitis

Finding	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MOD > 6 mm	93.2	8.6	49.7	33.3
Wall thickness > 3 mm	16.6	95.2	57.1	75
Periappendiceal fluid	61.4	76.8	75	64.7
Periappendiceal hyperechoic fat	58.1	64.9	77.3	43
Lymphadenitis	38.2	50.6	44.7	43.9
Appendicoliths	24.4	80.9	57.9	50
WBC count 11×10^9/l	84.3	28.2	55.1	63.1
CRP level > 100 mg/l	32.95	93.1	82.8	59.5

MOD – maximum outside diameter; WBC – white blood cells; CRP – C-reactive protein; PPV – positive predicted value; NPV – negative predicted value

of non-surgical treatment of perforated appendicitis are avoided. Even though computed tomography (CT) is still widely used as an imaging modality of choice for appendicitis [18, 19, 20], in our institution fewer than 5% of patients undergo CT for this diagnosis. As a radiation-free tool, operated by trained pediatric radiologists, ultrasonography is considered a method of choice in evaluating pediatric patients with suspected appendicitis [21, 22]. Additionally, some publications show poor correlation between CT reports of appendiceal perforation and intraoperative or histopathological findings [23].

In our study all 40 patients that had intraoperative or histopathological findings of perforation were classified as complicated appendicitis on ultrasonography. Moreover, we proved that all the patients that had perforation, had at least one of the following two ultrasonography parameters, periappendiceal fluid and hyperechoic periappendiceal fat. These results show high predictive value of the combination of these parameters as reported previously [11, 12]. On the other hand, when assessing the reports that were classified as uncomplicated appendicitis, we found that 43 of them had both of the aforementioned parameters negative, but only 32 of these patients (74.4%) had the diagnosis proved histopathologically. These results indicate that in order to rule out complicated appendicitis, some other factors should be included in the decision-making process. Most of the scoring systems, like the Alvarado score and appendicitis inflammatory response score, were developed to identify patients with appendicitis, discriminating it from non-appendicitis [24, 25]. Atema et al. [16] presented a scoring system based on seven clinical and ultrasonography features, with a cut-off value of six points, which showed high sensitivity of 95%, but low specificity of 45.7%. Düzgün et al. [26] presented a new scoring system based on the Alvarado score and diagnosed complicated appendicitis with sensitivity of 86.1% and specificity of 90.4% when patients had a score of 10.5 or higher.

Using the multivariate analysis, we were able to conclude that the interaction between periappendiceal fluid and hyperechoic periappendiceal fat on ultrasonography is significantly associated with complicated appendicitis. These results are consistent with the ones presented by Rawolle et al. [14] and Carpenter et al. [11]. Furthermore, we proved that two laboratory parameters, WBC count with cut-off value of 11×10^9/l and CRP of more than 100 mg/l, were also valuable predictors of complicated appendicitis as previously shown by Rawolle et al. [14]. Similarly, the sensitivity of 93.2% for appendiceal diameter greater than 6 mm, proved that it is a finding associated with complicated appendicitis, as stated in a recent paper by Bekiaridou et al. [12].

There are some limitations to our study, mostly related to its retrospective design. Because of the lack of standardization of the ultrasonography protocol, loss of the submucosal layer was not evaluated in all of our patients so we had to exclude that parameter from our statistical analysis. Due to these limitations, we are planning a prospective study with a bigger patient population where we would also include the loss of the submucosal layer as a predictive parameter. In addition, the size of our study population was affected due to the fact that patients who did not undergo appendectomy and received antibiotic-first treatment, were not included.

CONCLUSION

In conclusion, our results show that ultrasonography can be reliably used as a primary imaging modality for the differentiation between uncomplicated and complicated appendicitis in children, including gangrene and perforation. Beside the interaction between periappendiceal fluid and hyperechoic periappendiceal fat as sonographic parameters, also WBC count > 11×10^9/l and CRP > 100 mg/l proved to be significantly correlated to complicated appendicitis. Using these criteria, appendectomy can be avoided in a significant number of patients and those with uncomplicated appendicitis can be treated with antibiotics.

Conflict of interest: None declared.

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Употреба ултразвука и лабораторијских параметара у разликовању компликованог и некомпликованог апендицитиса код деце

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САЖЕТАК

Увод/циљ Циљ рада је био евалуирати ултразвучне и лабораторијске налазе као предикторе компликованог и некомпликованог апендицитиса у циљу доношења одлуке о даљим терапијским могућностима.

Методе У ову ретроспективну кохортну студију укључена су 174 педијатријска болесника у периоду од годину дана, којима су урађене лабораторијске анализе и ултразвучни преглед уочи апендектомије. Вршено је поређење резултата са интраоперативним и хистопатолошким налазом компликованог (гангренозног или перфоративног) и некомпликованог (флегмонозног) апендицитиса и рађена је процена уз помоћ бинарне логистичке регресије са елиминацијом уназад. Иницијални модел је укључивао осам предиктора. Након елиминације уназад, преостала су четири: периапендикуларна течност, хиперехогена периапендикуларна маст, број леукоцита и Ц-реактивни протеин (ЦРП). Коначни модел је укључивао и интеракцију између периапендикуларне течности и хиперехогене периапендикуларне масти. Дијагностичка вредност сваког параметра представљена је сензитивношћу и специфичношћу.

Резултати Од свих болесника, 86 је имало некомпликовани, а 88 компликовани апендицитис (37 гангренозни и 51 перфоративни). У коначном моделу три предиктора су била значајно повезана са компликованим апендицитисом: интеракција између периапендикуларне течности и хиперехогене периапендикуларне масти, број леукоцита > 11×10^9 / *l* и ЦРП > 100 *mg/l*. Укључивање интеракције између периапендикуларне течности и хиперехогене периапендикуларне масти их је искључило као индивидуалне предикторе. Највећу сензитивност од свих параметара (93,2%) показао је спољашњи дијаметар апендикса > 6 *mm*, док је најспецифичнији параметар (95,2%) била дебљина зида > 3 *mm*.

Закључак Коришћење периапендикуларне течности и хиперехогене периапендикуларне масти као ултразвучних предиктора, и броја леукоцита и ЦРП као лабораторијских предиктора, може да разликује некомпликовани од компликованог апендицитиса код деце и помогне клиничару да одлучи о терапији антибиотицима или хируршком третману.

Кључне речи: ултразвук; лабораторијски параметри; компликовани апендицитис; некомпликовани апендицитис; деца

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The impact of cycling exercise on motor and functional recovery of patients in acute and subacute stroke phase

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SUMMARY

Introduction/Objective Neurological impairment and immobility in stroke patients can lead to numerous complications. This study aimed to evaluate the effect of cycling exercises with visual feedback combined with conventional rehabilitation on neurological and motor recovery, balance, walking speed and endurance, and activities of daily living (ADL) in patients after acute and subacute stroke.

Methods A randomized prospective controlled trial was applied to this research. One hundred and twenty-seven hemiplegic stroke patients who received in-hospital rehabilitation were randomly assigned into two groups. Both groups received conventional rehabilitation treatment. The experimental group had an extra 30 minutes of cycling exercises for the upper and lower extremities on a stationary ergocycle MOTOmed muvi. Both groups' neurological status, upper and lower limb function, independence in ADL, balance, walking speed, and endurance were observed before and after the rehabilitation treatment. Outcome measures used were the National Institute of Health Stroke Scale (NIHSS), the modified Ashworth scale (MAS), the Brunnstrom Motor Evaluation Scale (BMES), upper and lower Fugl-Meyer assessment (FMA), the Barthel index (BI), the Berg Balance Scale (BBS), the six-minute walk test (6MWT) and the Timed Up and Go test (TUG).

Results The neurological recovery on the NIHSS scale, spasticity of the knee extensor measured by the MAS, the BMES and FMA-LE subscale for the affected leg, and the 6MWT presented more significant improvement in the experimental group than in the control group after the treatment ($p < 0.05$ for all three analyses).

Conclusion Cycling exercises with visual feedback combined with conventional rehabilitation could promote neurological recovery and improve the motor function of the affected leg and walking speed in patients recuperating after acute and subacute stroke.

Keywords: rehabilitation; stroke; hemiplegia; recovery of function; lower extremity

INTRODUCTION

Stroke represents the most frequent source of acquired disability in the adult population, which leads to reduced cognitive and motor functions and a decrease in patients' autonomy in activities of daily living (ADL). Stroke usually affects older adults and results from brain tissue injury caused by insufficient cerebral blood supply [1]. Hemiplegia is a prevalent symptom after acute stroke and the focus of rehabilitation. Additionally, many stroke survivors have impaired balance and mobility. Rehabilitation treatment after stroke is more effective if it is timely, intensive, and if it includes multisensory stimulation. Various rehabilitation approaches have been proposed, but few have been confirmed as effective in clinical research. The underlying mechanism of neurological deficit recovery after stroke is still not fully explained because more than one process is involved in recovery, and cerebral plasticity plays a significant role [2]. Stationary ergocycle is uncomplicated and provides inexpensive exercise that improves muscle strength, stamina, and balance [3]. MOTOmed muvi is a new stationary ergocycle with different exercise modes. It allows recording and provides essential information on the patient's improvement in real time. That way, it can assist the clinician and therapist determine optimal training intensity and frequency to promote recovery [4]. However, there has been an insignificant number of clinical studies that examined the impact of cycling exercises on the recovery of hemiplegic patients during the acute and subacute stroke phases.

This study aimed to determine the effect of the cycling exercises performed on stationary ergocycle as an addition to conventional rehabilitation on neurological and motor recovery, balance, ADL, and walking speed and endurance in patients after acute and subacute stroke.

METHODS

Our research was devised as a prospective randomized controlled trial. The participants were

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stroke patients receiving in-hospital rehabilitation at the Medical Rehabilitation Clinic, Clinical Centre of Vojvodina from 01. March 2022 to 31. April 2023. The study was approved by the ethics committee of the Clinical Centre of Vojvodina (Ref. No. 2022-600-184). All study participants gave their written informed consent. The inclusion criteria were as follows: first stroke; stroke onset less than three months; diagnosis of ischemic or hemorrhagic stroke based on neurological examination, brain computed tomography (CT), or magnetic resonance imaging (MRI); unilateral hemiplegia; initial National Institute of Health Stroke Scale (NIHSS) score ranging 6–20; age of more than 18 years; cognitive ability to participate in rehabilitation treatment; minimal ability to actively perform movements in the shoulder and elbow with compensatory trunk movement. The exclusion criteria were recurrent stroke, stroke accompanied by severe mental disorder, patients with aphasia who could not comply with directions, and heart, liver, or renal failure. The calculation of the test power was carried out for the research. The sample size was calculated using the software G*power 3.0.10 [5]. We used an alpha level of $p \leq 0.05$, a study power of 0.8, and an effect level of 0.15 (small effect size). The sample size amounted to 90 subjects (45 each in each group)f or the combined analysis of variance [6].

After baseline assessment, eligible patients who met the inclusion criteria were randomly assigned to the experimental and control group in a 1:1 ratio. Computer-generated numbers were used for randomization. The numbers were stored in sealed envelopes, handled by a physician unaware of the study's purpose. The resident physician (T.S.) created the random allocation sequence using the EpiDat v. 4.0 software and maintained it classified so that allocation remained concealed. When patients appeared for the first rehabilitation session, the assigned therapist started the randomization within the computer program to irrevocably designate the patient to the control or experimental group prior to disclosing the procedure.

Both groups underwent regular conventional in-patient rehabilitation treatment for three weeks, six days per week (18 sessions). Conventional rehabilitation consisted of physical and occupational therapy, each lasting one hour. Physical therapy involved personalized exercises chosen by the therapist, manual mobilization, and physical agents. Occupational therapy implemented repetitious exercises to improve coordination and ADL skills using different standard equipment. The experimental group had an additional 30 minutes of cycling exercises for the upper and lower extremities with visual feedback on a stationary ergocycle (MOTOmed muvi, RECK-Technik, Betzenweiler, Germany). MOTOmed muvi ergocycle enables simultaneous leg and arm training. The ergocycle panel showed parameters of symmetry of bilateral upper and lower limb exertion, cycling extent (in kilometers), achievement (watts), resistance (kilograms), and number of revolutions per minute (rpms). The data during cycling were recorded on a computer.

The cycling exercises for the upper and lower extremities consisted of 15 minutes forward and 15 minutes backward movement. Every training began with preparation; patients were seated on a chair before the stationary ergocycle. Heart rate and arterial tension were measured at each session's beginning and end. Preparation was followed by passive warm-up: 150 seconds of passive cycling exercises so that the ergocycle moved the arms and legs of the patient at a steady pace of 25 rpm. After warm-up, the patients started active cycling exercise, which consisted of 10 minutes of active cycling for arms and legs. They were instructed to maintain a pedaling speed of 50–70 rpm. Visual feedback was used to accomplish load symmetry 50/50 on the ergocycle panel. The weight of active exercise was settled as Stage 13 of the Borg scale [7], signifying "a little strenuous" training. The session ended with passive training: 150 seconds of passive cycling exercises, the patient's limbs were moved by the ergocycle at a steady pace of 25 rpm.

The patients' neurological and functional status was assessed at the baseline (within the first 24 hours of admission to the Clinic) and after 18 therapy sessions. The assessment was carried out by the specialist of physical medicine and rehabilitation (S.P.), who was unaware whether the patients were assigned to the control or experimental group. NIHSS was used to estimate neurological impairment [8]. The Modified Ashworth Scale (MAS) was used to determine the knee extensor's spasticity level [9]. The Berg Balance Scale (BBS) was used to evaluate static balance and fall risk. It evaluates balance during activities such as standing, sitting, transfers, and rotations needed in ADL. A higher score implies better balance and reduced fall risk; the best result is 56 points [10]. Motor function was classified by the Brunnstrom Motor Evaluation Scale (BMES) [11] for the hemiparetic arm, hand, and leg. It has six stages: the first one is characterized by flaccidity and the inability for voluntary movement, and the last one is achieved when the patient performs isolated joint movement. BMES is a frequently administered stroke-specific tool for determining the post-stroke motor recovery level and gross hemiparesis severity [11, 12]. However, it is subjective, and due to rough evaluation, minor functional changes in recovery can be overlooked [12]. Because of these limitations, we also applied the Fugl-Meyer assessment (FMA) for a more detailed examination. FMA is based on BMES but has more sensitivity for subtle changes in motor recovery and is responsive and feasible [13, 14]. FMA analyses the reflex activity of the affected extremities, movements, and their relation to synergies, speed, and coordination. We used subsections of FMA for the upper (FMA-UE) and lower extremity (FMA-LE). FMA-UE incorporates 33 items for proximal and distal segments of the affected arm with a maximum motor score of 66 points. The FMA-LE subscale has 17 items; the highest score of 34 points is received for normal function [14]. Barthel index (BI) was applied to evaluate independence in ADL. The BI score estimates 10 essential activities needed for self-care and mobility [15]. The maximum result is 100, and lower results mean that the patient suffers from a more remarkable inability to perform ADL without help. The six-minute walk test (6MWT) and the Timed up and Go test (TUG) were used to analyze walking speed and endurance [16].

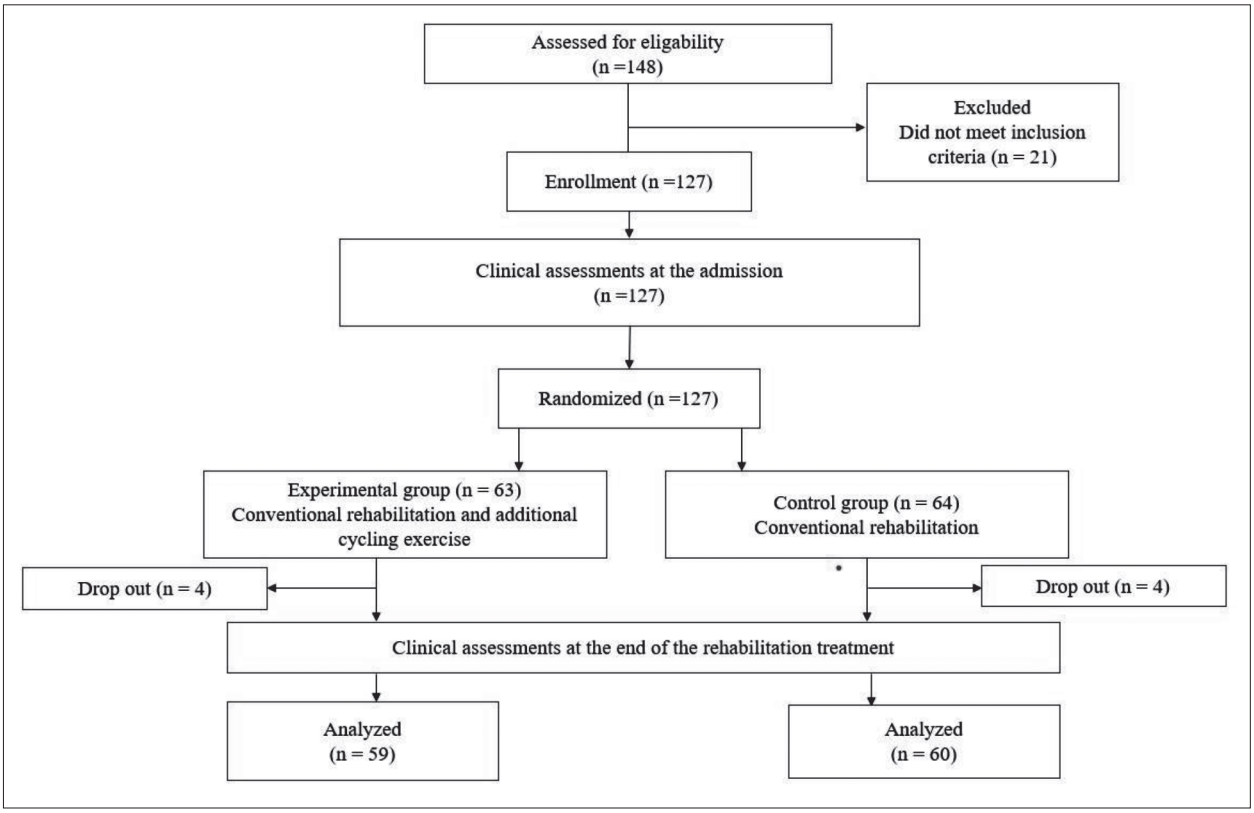

Figure 1. Flowchart of the design and conduct of the study

Statistical analysis

In our study, IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) statistical software was applied for data processing and analysis. Frequency and percentage were used to describe the sample structure. Descriptive statistics methods were used to determine measures of central tendency (arithmetic mean) and measures of variability (standard deviation) of observed clinical data. The patients' demographic and clinical baseline characteristics in the two groups were compared using the χ^2 test for categorical variables, the independent t-test for continuous variables, and the Mann–Whitney U test for ordinal variables. The assessments at the beginning and end of each group's rehabilitation treatment were compared to determine if there were changes after the administered therapy. A Student's t-test analyzed quantitative variables. To estimate the treatment effect and differences between the control and experimental groups in two-time intervals (the beginning and the end of treatment), a split-plot analysis of variance (SPANOVA) was used. The significance level was set at $p < 0.05$.

RESULTS

The recruitment interval for this study lasted from March 1, 2022 to April 31, 2023. The participant flow is described in Figure 1. A total of 127 patients were recruited. Eight patients left the protocol for several reasons, such as illness unrelated to the study, personal reasons, and loss of desire to participate in the prescribed treatment due to the development of severe depression. The rehabilitation treatment was completed by 119 patients (Figure 1). Table 1 summarizes participant demographics. Participants in this study had moderate neurological deficits, evaluated by the NIHSS scale. Demographic and clinical data of the two groups were compared at initial evaluation, and no substantial differences were detected between groups (Table 1).

Table 1. Demographic and clinical characteristics of patients

Variables	Experimental group (n = 59)	Control group (n = 60)	p
Sex (male/female)	31/28	29/31	0.71
Age (years)	65 ± 11.98	67.34 ± 10.86	0.84
Type of stroke (I/H)	49/10	53/7	0.67
Side of hemiplegia (left/right)	24/35	28/32	0.41
Time since stroke (days)	33 ± 17.2	36 ± 19.7	0.89
NIHSS (0–42)	10.3 ± 4.45	11.4 ± 5.67	0.63

Data are presented as mean ± SD;
I – ischemic; H – hemorrhagic; NIHSS – National Institute of Health Stroke Scale

The values of clinical assessments for the experimental and control groups at the initial evaluation and the end of rehabilitation treatment are shown in Table 2. No significant difference among the experimental and control groups was observed at the first assessment.

Patients in the experimental group showed considerable improvement in all parameters of the stationery ergocycle after 18 sessions. The average cycling extent (distance) progressed from 2612 ± 454 meters measured at

Table 2. Values of outcome measures at baseline (T1) and the end of rehabilitation treatment (T2)

Outcome measures	Group	T		Change
		T1	T2	
NIHSS (0–42)	Experimental	10.3 ± 2.29	8.0 ± 2.14	-2.3 ± 0.95
	Control	11.4 ± 3.09	9.5 ± 3.26	-1.9 ± 1.26
	p-value	0.438	0.227	*0.030
MAS	Experimental	1.8 ± 0.79	0.6 ± 0.52	-1.2 ± 1.05
	Control	2.0 ± 1.10	1.8 ± 1.31	-0.2 ± 0.75
	p-value	0.826	*0.002	*0.003
BMES arm (1–6)	Experimental	4.2 ± 1.34	4.8 ± 1.25	0.6 ± 0.57
	Control	3.7 ± 1.31	4.5 ± 1.46	0.8 ± 0.50
	p-value	0.372	0.413	0.740
BMES hand (1–6)	Experimental	4.2 ± 1.41	4.7 ± 1.39	0.5 ± 0.75
	Control	3.8 ± 1.51	4.4 ± 1.64	0.6 ± 1.22
	p-value	0.286	0.425	0.820
BMES leg (1–6)	Experimental	4.3 ± 1.03	5.2 ± 1.03	0.9 ± 1.21
	Control	4.0 ±1.09	4.5 ± 1.15	0.5 ± 0.33
	p-value	0.553	*0.047	*0.049
FMA–UE (0–66)	Experimental	35.2 ± 15.72	42.0 ± 16.27	6.8 ± 2.05
	Control	33.6 ± 21.17	39.2 ± 21.80	5.6 ± 4.58
	p-value	0.641	0.429	0.073
FMA–LE (0–34)	Experimental	21.0 ± 5.42	29.5 ± 4.79	9.0 ± 2.75
	Control	20.2 ± 7.84	24.3 ± 7.86	4.1 ± 1.41
	p-value	0.556	*0.000	*0.000
BI (0–100)	Experimental	46.9 ± 19.09	64.8 ± 21.1	17.9 ± 15.71
	Control	51.1 ± 17.29	65.8 ± 17.8	14.7 ± 13.75
	p-value	0.371	0.653	0.239
BBS (0–56)	Experimental	28.6 ± 9.79	43.3 ± 8.64	14.7 ± 3.94
	Control	29.9 ± 13.82	39.2 ± 13.08	9.32 ± 2.83
	p-value	0.543	*0.048	*0.000
6MWT (meters)	Experimental	159.8 ± 78.62	241.6 ± 94.54	81.8 ± 70.96
	Control	142.4 ± 93.33	174.8 ± 87.12	32.4 ± 26.75
	p-value	0.293	*0.032	*0.035
TUG (seconds)	Experimental	96.2 ± 64.94	131.7 ± 84.43	35.5 ± 30.57
	Control	102.6 ± 72.42	121.9 ± 97.33	24.6 ± 19.21
	p-value	0.594	0.136	0.092

Data are presented as mean ± SD;
NIHSS – National Institute of Health Stroke Scale; MAS – modified Ashworth scale; BMES – Brunnstrom Motor Evaluation Scale; FMA-UE – Fugl-Meyer upper extremity subscale; FMA-LE – Fugl-Meyer lower extremity subscale; BI – Barthel index; BBS – Berg Balance Scale; 6MWT – six-minute walk test; TUG – Timed up and Go test;
*p < 0.05 is significant

Table 3. Split-plot analysis of variance (SPANOVA) for outcome variables

Variable	Wilks' λ	F	p	Partial η^2
NIHSS				
Time (beginning vs. end)	0.225	403.139	*0.000	0.775
Time* group	0.966	4.148	*0.044	0.034
Experimental vs. Control		7.045	*0.009	0.057
MAS				
Time (beginning vs. end)	0.886	312.436	*0.000	0.754
Time* group	0.019	2.369	0.086	0.031
Experimental vs. Control		5.842	*0.022	0.083
BMES arm				
Time (beginning vs. end)	0.513	110.316	*0.000	0.487
Time* group	0.976	2.889	0.092	0.024
Experimental vs. Control		2.437	0.121	0.021
BMES hand				
Time (beginning vs. end)	0.509	111.992	*0.000	0.491
Time* group	0.983	2.022	0.158	0.017
Experimental vs. Control		2.328	0.130	0.020
BMES leg				
Time (beginning vs. end)	0.494	118.75	*0.000	0.506
Time* group	0.973	3.269	0.073	0.027
Experimental vs. Control		4.634	*0.041	0.076
FMA-UE				
Time (beginning vs. end)	0.244	361.945	*0.000	0.756
Time* group	0.973	3.270	0.073	0.027
Experimental vs. Control		0.408	0.524	0.003
FMA-LE				
Time (beginning vs. end)	0.098	1071.563	*0.000	0.902
Time* group	0.438	149.923	*0.000	0.562
Experimental vs. Control		7.036	*0.009	0.047
BI				
Time (beginning vs. end)	0.772	304.326	*0.000	0.633
Time* group	0.024	2.899	0.091	0.010
Experimental vs. Control		0.548	0.446	0.005
BBS				
Time (beginning vs. end)	0.074	1466.058	*0.000	0.926
Time* group	0.612	74.272	*0.000	0.388
Experimental vs. Control		0.425	0.516	0.004
6MWT				
Time (beginning vs. end)	0.686	112.325	*0.000	0.521
Time* group	0.922	4.699	0.062	0.112
Experimental vs. Control		6.862	*0.033	0.032
TUG				
Time (beginning vs. end)	0.621	213.345	*0.000	0.578
Time* group	0.054	2.683	0.073	0.018
Experimental vs. Control		3.203	0.062	0.065

NIHSS – National Institute of Health Stroke Scale; MAS – modified Ashworth scale; BMES – Brunnstrom Motor Evaluation Scale; FMA-UE – Fugl-Meyer upper extremity subscale; FMA-LE – Fugl-Meyer lower extremity subscale; BI – Barthel index; BBS – Berg Balance Scale; 6MWT – six-minute walk test; TUG – Timed up and Go test;
*p < 0.05 is significant

the initial session to 3978 ± 868 meters at the final session ($p < 0.001$). The mean achievement (wattage) raised from 13.7 ± 6.73 watts at the first session to 26.8 ± 10.36 watts at the final session ($p < 0.001$). The average resistance at the initial session was 5.8 ± 2.45 kg, which advanced to 8.6 ± 3.89 kg at the final session ($p < 0.001$).

At the end of the treatment (discharge), the experimental group presented more pronounced neurological recovery on the NIHSS scale, reduced spasticity of the knee extensor measured by the MAS, more substantial improvement on the BMES for the affected leg, the FMA-LE subscale, the BBS, and 6MWT ($p < 0.05$).

We used SPANOVA to determine the impact of two therapeutic approaches. The results of the SPANOVA analysis confirmed a statistically significant difference in the neurological recovery measured by the NIHSS scale ($F_{1, 117} = 7.045$, $p = 0.009$), spasticity of the knee extensor measured by MAS ($F_{1, 117} = 5.842$, $p = 0.022$), the BMES for the affected leg ($F_{1, 117} = 4.634$, $p = 0.041$), FMA-LE subscale ($F_{1, 117} = 7.036$, $p = 0.009$) and 6MWT ($F_{1, 117} = 6.862$, $p = 0.033$) at the end of rehabilitation treatment in favor of the experimental group (Table 3). For all tested variables, changes between the pretest (beginning of the rehabilitation treatment) and posttest values (end of rehabilitation

treatment) in both groups were highly statistically significant ($p < 0.001$). The effect size was large; thus, both groups of patients benefited from rehabilitation treatment. The interaction between the treatment type and time is shown in Table 3.

DISCUSSION

Various studies have shown that brain function is, to a degree, compensatory after stroke [17, 18]. Based on neuroplasticity, the function of the central nervous system can be improved by intensive rehabilitation. Furthermore, timely applied treatment can enhance the gene expression of nerve growth factors, improve neurotransmitter transmission, and advance motor function [19]. Current research findings imply that aerobic training can improve the strength of affected limbs, balance, and walking speed after stroke [20]. Stationary ergocycle MOTOmed muvi provides passive, assisted, and active resistance training modes for the upper and lower extremities. The used mode can be adapted for every patient based on the motor recovery stage. Cycling exercises prevent muscle atrophy, expand the range of joint motion, and help patients gain confidence to participate in the rehabilitation treatment [21]. Reports agree that most of the recovery after a stroke occurs within the first 3–6 months [22, 23]. However, there is limited research on the impact of cycling exercise on motor improvement during the acute and subacute stroke phases. In our study, we noted a more substantial neurological recovery assessed by the NIHSS in the experimental group, although the interaction between the type of treatment and time was significant. Máté et al. [22], in their meta-analysis, confirmed that cycling exercise combined with functional electrical stimulation can enhance neurological recovery and aerobic fitness in patients with central nervous system disorders. Wei et al. [23] found that early rehabilitation positively affects neurological recovery measured by the NIHSS after stroke. The results of the present study demonstrated that the experimental group displayed significantly lower spasticity at discharge, similar to some other studies [18, 20]. This might be because visual feedback cycling exercises activate the monosynaptic corticospinal inhibition pathways and reduce the transmission from neurons to muscles, reducing muscle spasticity [24]. Our findings show that after the treatment, the experimental group exhibited more prominent improvement in BMES for the affected leg, FMA-LE subscale, and 6MWT, indicating that cycling exercises can substantially improve lower limb motor and walking functions. Similar results were obtained in previous studies, which evaluated the use of cycling training in chronic stroke [24, 25]. Nindorera et al. [26], in their research, combined conventional rehabilitation and cycling training for patients with chronic stroke and discovered that applied protocol could improve lower extremity function and stamina, gait speed, and reduce muscle tone. Furthermore, the patterns of muscle activity during walking and cycling require alternate flexion and extension motion and corresponding activation of agonist and antagonist musculature. This can benefit neuromuscular regulation and muscle activation of the paretic lower extremity [27]. In our research, the upper extremity motor function (the BMES for the affected arm and hand and the Fugl-UE subscale) significantly increased after rehabilitation treatment. Nevertheless, the difference among the groups after the treatment was insignificant ($p > 0.05$). In their study, Linder et al. [24] obtained similar results. Despite the significant change in BI for both groups after the treatment, our findings imply that both treatments were equally beneficial. This is in accordance with several other trials [22, 25, 27]. Considering the effect of additional cycling exercise on balance (BBS), we found no statistically discernible difference among the groups, although the experimental group presented a higher trend of improvement. Duran et al. [27], in their research, found that different treatment approaches had a modest impact on balance in stroke patients (cycling training *vs.* underwater walking therapy *vs.* conventional rehabilitation). Our results confirm that cycling exercises as part of a post-stroke rehabilitation program can promote lower limb recovery, reduce spasticity, and improve gait recovery.

The limitations of this study include the difference in the duration of treatment for the examined groups, which burdens the generalization of the results. More tests could be applied to monitor hand functions aside from the FMA-UE, which could be the topic of further research. Research with longer and more rigorous follow-ups is needed to examine the long-term benefits of cycling exercises for stroke patients. Furthermore, this study was carried out in a single rehabilitation clinic with patients who mostly suffered from moderate stroke, so our findings may not be suitable for all settings.

CONCLUSION

Our results imply that cycling exercises on a stationary ergocycle combined with conventional rehabilitation could improve neurological and motor recovery of hemiparetic lower extremity and walking speed in acute and subacute stroke patients. Cycling exercises with visual feedback could be part of a protocol for the in-hospital rehabilitation of acute or subacute stroke patients.

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Утицај вежби на стационарном ергоциклу на моторички и функционални опоравак болесника у акутној и субакутној фази можданог удара

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САЖЕТАК

Увод/Циљ Неуролошки дефицит и смањена покретљивост код болесника са можданим ударом могу довести до бројних компликација. Ова студија је имала за циљ да процени ефекат вежби у виду *feedback* тренинга ергоциклом у комбинацији са конвенционалном рехабилитацијом на неуролошки и моторички опоравак, баланс, брзину хода, издржљивост при ходу и активности свакодневног живота код болесника после акутног и субакутног можданог удара.

Методе Ово истраживање дизајнирано је као рандомизирана проспективна контролисана студија. Сто двадесет седам болесника са хемиплегијом после можданог удара који су били на болничкој рехабилитацији рандомизирано је у две групе. Обе групе су имале конвенционални рехабилитациони третман. Експериментална група је добила додатних 30 минута вежби за горње и доње екстремитете на стационарном ергоциклу *MOTOmed muvi*. Неуролошки статус, функција горњих и доњих екстремитета, независност у активности свакодневног живота, баланс као и брзина и издржљивост при ходу, процењени су пре и после рехабилитације код обе групе. Коришћене скале за процену исхода биле су скала Националног института за здравље за мождани удар (*NIHSS*), модификована Асхвортова скала (*MAS*), *Brunnstrom* скала (*BMES*), *Fugl-Meyer* процена за горње и доње екстремитете (*FMA*), Бартел индекс (BI), Бергова скала баланса (*BBS*), шестоминутни тест хода (6*MVT*) и тест „устани–крени" (*TUG*).

Резултати Неуролошки статус процењен на основу скале *NIHSS*, спастичност екстензора колена мерена помоћу *MAS*, *BMES* и *FMA-LE* субскала за захваћену ногу, као и 6*MWT* показали су значајно веће побољшање у експерименталној групи у односу на контролну групу после третмана ($p < 0,05$ за све три варијабле).

Закључак Вежбе на ергоциклу са визуелним *feedback*-ом у комбинацији са конвенционалном рехабилитацијом могу унапредити неуролошки опоравак, побољшати моторичку функцију захваћеног доњег екстремитета и брзину хода код болесника после акутног и субакутног можданог удара.

Кључне речи: рехабилитација; мождани удар; хемиплегија; функционални опоравак; доњи екстремитет

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Human resource management and community health services outcome – unravelling relationships in public healthcare organizations

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SUMMARY

Introduction/Objective Human resource management and related practices represent a broad research arena in the context of healthcare organizations. Adapting human resource management practices to current labor market conditions and achieving organizational goals in the domain of satisfactory health service quality represent significant current challenges and research gap. The aim of the study is to examine the relationship between human resource management practices and health service outcomes of public healthcare organizations.

Methods The research was designed as a cross-sectional study. A structured questionnaire was used as an instrument to collect primary data. The sample consists of 257 healthcare workers employed in healthcare organizations in the public sector. To assess the statistical significance of relationships in the research model, the method of PLS-SEM is used.

Results Our study revealed the competitive salary is negatively related to community health service results (CHSR) ($\beta = -0.177$, $p < 0.05$) of public healthcare organizations. Study results noted that employment security is positively related to CHSR ($\beta = 0.296$, $p < 0.001$), as well as to training and development ($\beta = 0.359$, $p < 0.001$).

Conclusion The results of the study noted theoretical implications through the contribution of human capital theory and resource-based theory of the firm. The identification of human resource practices that positively contribute to health services outcomes provides clear practical implications for managers of public health organizations.

Keywords: HRM; HRM practice; health services outcome; organizational performance

INTRODUCTION

Human resource management (HRM) in healthcare organizations involves a unique set of practices designed to enhance organizational performance through the actions of healthcare personnel in interaction with patients. The concept of HRM in healthcare is critical because it directly impacts the operational efficiency of healthcare organizations [1], the quality of care provided, and can affect patient satisfaction. HRM has been identified as a critical success factor of any healthcare system and contributes to its sustainability [2, 3]. The practice of HRM supports the establishment of healthcare organizations' business models through a variety of activities, including employee relations, compliance and legal matters, recruitment and selection (RS), training and development (TD), and performance management. Each of the mentioned activities has a specific importance for operational efficiency, which is achieved by applying appropriate approaches and procedures in interaction with healthcare personnel. TD focus on providing ongoing education and training to healthcare workers to enhance their skills, competences, and knowledge. Fanelli et al. [4] reached the conclusion that managerial and clinical competencies hold equivalent significance for healthcare professionals. The aforementioned development of the competencies establishes the background for improving performance and contributes in the development of the HRM system architecture within healthcare organizations. RS practices ensure that the organization hires skilled and competent staff, including doctors, nurses, and support staff. Successful recruitment of healthcare providers requires face-to-face meetings, building relationships with administrators, and utilizing multimodal strategies simultaneously [5]. Performance management ensures that healthcare professionals meet the organization's care delivery standards and adhere to medical protocols. Newton-Lewis et al. [6] suggested the performance management in healthcare organizations is crucial for driving organizational and system performance, but interventions should be tailored to the specific health system context. Chapman [7] noted that performance management system is crucial for strengthening health departments and effectively using resources, as it is a key factor in their long-term sustainability. A system of caring HRM practices, such as job design, training, flexible work arrangements, work-life balance,

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and participation in decision making, results in an organizational climate of care and concern for employees, leading to higher levels of employee engagement [8]. The results of the research conducted by Sivapragasam and Raya [9] showed that HRM practices have a statistically significant relationship with employee-level outcomes like perceived efficacy, engagement, and well-being. The proliferation of digitization and the rapid advancement of information technology have empowered platforms and information systems to provide an extensive array of functionalities that facilitate HRM activities. A human resources information system (HRIS) enables the deployment of HRM practice more efficiently and productively by digitizing procedures and activities. On the other hand, Tursunbayeva [10] suggested that healthcare organizations and HR professionals employed within need to use HRIS responsibly, finding a balance between innovation, productivity, efficiency, and respect for legal, ethical, and compliance issues.

The importance of HRM for healthcare organizations is profound and multifaceted, deeply influencing every aspect of healthcare delivery. The direct effect of HRM practices is often complemented by positive indirect effects that are realized in different contexts. Ranjhan and Mallick [11] have found the HRM practice significantly moderates the positive relation between organizational citizenship behavior and competitive advantage in healthcare organizations. HRM practice appears as essential for developing competencies in healthcare professionals, ensuring the recruitment of skilled staff, and maintaining high standards of care delivery.

HRM and healthcare organization performance

The beneficial effects of HRM practices on various aspects of healthcare organizations' performance have been substantiated by a multitude of prior studies. Vermeeren et al. [12] found that HR policies positively affect the financial performance of healthcare organizations, minimize illness absence, and enhance organizational performance associated with patients. Van den Broek [13] suggests that employees play an intermediary role in the relationship between HRM and organizational performance. Therefore, HR practices should simultaneously consider both enhancing organizational performance as well as upholding employees' work–life balance. Based on a cross-section study, Nafari and Rezaei [14] noted the positive impact of HRM activities on the organizational performance of healthcare organizations. Using regression models, they came to the conclusion that statistically the most significant impact is achieved by HR practices related to TD, compensation and reward, and recruitment. Huettermann and Bruch [15] revealed the health-related HRM has a positive effect on the well-being of employees, as well as having a positive indirect impact on organizational performance. Buchelt et al. [16] suggest that healthcare organizations should transform HR practices so that performance evaluation includes both quantitative and qualitative dimensions. Important conclusions about the impact of HRM on the organizational performance of hospitals were discovered by Parayitam et al. [17]. The results of their research showed that compensation and rewards, performance appraisal (PA), and learning culture have a positive impact on organizational performance. Also, it was revealed that RS, and TD have no influence on the hospital's organizational performance. Acosta-Prado et al. [18] noted that HRM strongly predicts innovative performance of non-profit hospitals, while innovation can also enhance organizational performance by acting as a mediator, according to Riana et al. [19], who found that HRM practice has a substantial impact on both innovation and organizational performance.

These studies suggest that HRM practices are related to improved performance in healthcare organizations, including employee satisfaction, and organizational efficiency. This establishes a framework for enhancing the quality of health services as a fundamental purpose.

HRM and health service outcome

Healthcare organizations may observe a diverse array of effects as a result of their HRM practices. The realization of the multidimensional effects of human resource practice occurs via strategic alignment, which entails guiding and leading employees to work in the direction of organizational goals and operational efficiency. Implementing HR practices at both the strategic and operational levels is essential for healthcare organizations to achieve highest level of positive outcomes. This approach is based on the argument that retaining and satisfying employees positively influences the quality of care, leading to increased patient satisfaction.

HR practices recorded a direct and positive effect on client satisfaction, according to an important finding of the research conducted by Vermeeren et al. [12]. HR capabilities have a positive and statistically significant relationship with proactive healthcare worker behaviors and a beneficial association with patient care, according to a study by Khatri et al. [20]. Furthermore, proactive healthcare worker behaviors mediate the aforementioned relationship. The aforementioned study well illustrates the connection between HR practices and health service outcomes, indicating the valorization of its effects through the behavior of employees in healthcare organizations. A research study by Oppel et al. [21] showed that investments in strategic HRM are reasonable because they have a positive effect on patient satisfaction using two mechanisms: solving physician shortage problems and reducing temporary staffing. Limited implementation of adequate human resources practices can have far-reaching consequences beyond mere diminished patient satisfaction. HR practices positively impact health service outcomes, with engagement serving as a mediating factor, according to a study by Shantz et al. [22]. Every single HR practice examined in the aforementioned study, namely communication, participation in decision making, training, opportunities for development, and training, had a positive effect on the quality of care. This finding underscores the importance of implementing strategic HRM to effectively manage personnel and guide activities.

A significant impact of HRM practices on health service outcomes can be identified. Summarizing the above, healthcare organizations have to establish HR practices that are patient-centric and prioritize service quality and patient satisfaction. The primary objectives of strategic HRM are endeavors to enhance patient-provider communication, programs to improve the patient experience, and staff training in customer service. These practices are able to ensure that the healthcare professionals are oriented towards providing high-quality patient care. The aim of the paper is to identify elements of HR practice that can have a positive effect on health service outcomes.

METHODS

Participants and procedure

A cross-sectional study was conducted to determine the statistical significance of the relationship between HRM and health services outcomes. The study involved the participation of healthcare professionals employed by healthcare organizations located in the central region of the Republic of Serbia, including Belgrade as the capital. The research sample consisted exclusively of personnel employed by public healthcare organizations. Responses were obtained at an 85% rate, as 257 valid questionnaires were collected in total from 300 that were distributed. Women comprise the majority of the study participants at 84 percent, with the remaining individuals being males. The sample primarily consists of healthcare personnel who are under the age of 40, comprising 53% of the participants. Those aged 41 to 50 are represented at 30%, and those aged 51 and above are represented at 17%. A total of 86% of the respondents in the sample hold permanent employment status, while the remaining individuals are temporary staff members. The sample structure guarantees the essential diversification amidst a homogeneous group of healthcare professionals who are employed by public healthcare organizations.

The study was approved by the Council of the Faculty of Medical Science, University of Kragujevac, on Jun 22, 2022 (reference number 01-7218/18-52). We collected primary research data in accordance with the Declaration of Helsinki ethical guidelines. All participants were carefully apprised of the scientific objectives of the research and were assured of their anonymity. Informed written consent was noted on the first page of the questionnaire. Participants were notified that participation was voluntary, and their consent was inferred upon finishing the questionnaire. Data confidentiality was also guaranteed.

Several assumptions were implemented to mitigate the issue of common method bias. Prior to the study, all possible participants were briefed about the academic purpose of the study and guaranteed anonymity and confidentiality. Additionally, detailed directions for completing the questionnaire were provided at the start of each section. Finally, in the questionnaire itself there are clearly separated sections that contain statements that observe independent variables, dependent variable, and categorical variables.

Measurements

Primary data were gathered through the use of a structured questionnaire comprising three distinct sections. The statements containing the constructs of the independent variable were positioned in the initial section. Observations of the dependent variable were detailed in the statements forming the second section. In both sections, next to the column in which the statements were listed, there was a scale for evaluating the statements. At the end of the questionnaire, there were statements with categorical variables, such as gender, age, and employment status of the respondents. The items in the first two sections were evaluated on a five-point Likert scale.

To enhance the predictability of the research and minimize the risk of an initial error during questionnaire development, we opted for an approach that utilized standardized measurement scales. Such scales are present in previous studies through which their validation was carried out and their utility value was confirmed. We translated the measuring scales from English to Serbian before using them. In the second step, through a pilot study on a sample of 30 respondents, their validity was tested through the analysis of Cronbach's alpha coefficient. The results of the pilot study showed that the necessary criteria were met, after which the sampling continued. The following measurement scale was deployed within the study.

We measured the independent variable through six constructs that represent HR practice. The HRP scale developed by Villajos et al. [23] included constructs such as TD, contingent pay and rewards (CPR), PA, RS, competitive salary (CS), and employment security (ES). Each construct was observed using three statements, such as, *The opportunity to receive training and attend courses and workshops*, *A benefits and rewards plan that is linked to my performance*, *A fair evaluation of my performance*, *Careful selection of new employees*, and *A work contract that offers job security*.

The dependent variable was observed through the Community Health Services Results construct. In our study, it consists of statements initially noted within the Baldrige Health Care Criteria by Meyer and Collier [24]. The two items were used: *Contribution to community health programs*, and *Partnership with other organizations to improve community health programs*.

The creation of the original data set and preliminary statistical analyses were done using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). To test the relationship between HRM practices and health service outcomes, a partial least square approach to structural equation modeling (PLS-SEM) was used. Complex relationships between variables have been effectively modelled using the aforementioned method, even when normality criteria are not met or the sample size is relatively small. The outcomes for the validation of the measurement model were acquired through the employment of confirmatory factor analysis. In order to estimate the structural model and analyze the path coefficients, the standard bootstrapping procedure was applied.

RESULTS

The standard PLS-SEM algorithm procedure was launched to measure model assessment. The outcomes of the confirmatory factor analysis were used as the framework for testing the reliability and validity of measurement scales and statements. The internal consistency reliability and convergent validity analysis results of the proposed model are displayed in Table 1. Standard coefficients and benchmark values were used for evaluation. Factor loadings are significantly above the value of 0.7 for all statements contained in the constructs, which confirms their validity. For all the constructs contained in the model, the value of Cronbach's alpha (α) coefficient ranges 0.830–0.955 and largely meets the requested criteria. The composite reliability value ranges 0.892–0.956 for all latent variables and is significantly above the 0.7 standardized criterion. Average variance extracted is represented by values 0.733–0.918 and is significantly higher than the acceptable threshold of 0.5 value [25].

Table 1. Measurement model and constructs

Construct and items	Loadings	α	CR	AVE
TD		0.955	0.956	0.917
TD01	0.957			
TD02	0.965			
TD03	0.950			
CPR		0.932	0.936	0.880
CPR01	0.913			
CPR02	0.960			
CPR03	0.940			
PA		0.952	0.952	0.912
PA01	0.938			
PA02	0.970			
PA03	0.956			
RS		0.953	0.954	0.914
RS01	0.958			
RS02	0.967			
RS03	0.943			
CS		0.830	0.892	0.733
CS01	0.859			
CS02	0.802			
CS03	0.905			
ES		0.918	0.926	0.859
ES01	0.907			
ES02	0.948			
ES03	0.924			
CHSR		0.911	0.911	0.918
CHSR01	0.957			
CHSR02	0.959			

TD – training and development; CPR – contingent pay and rewards; PA – performance appraisal; RS – recruitment and selection; CS – competitive salary; ES – employment security; CHSR – community health services results; AVE – average variance extracted; CR – composite reliability

The Fornell–Larcker criterion was used for analysis and conclusions about discriminant validity. The results of the analysis shown in Table 2 show that the initial value in each column is higher than any other value in the same column, that comply with the Fornell–Larcker criterion. By validating statements and measurement scales, assumptions were made for starting the standard bootstrapping procedure and testing the relationships between the constructs.

Table 2. Discriminant validity (Fornell–Larcker criterion)

Constructs	1	2	3	4	5	6	7
1. CHSR	0.958						
2. CS	0.526	0.856					
3. CPR	0.599	0.782	0.938				
4. ES	0.670	0.686	0.606	0.927			
5. PA	0.658	0.795	0.822	0.695	0.955		
6. RS	0.658	0.803	0.716	0.738	0.868	0.956	
7. TD	0.727	0.607	0.685	0.718	0.761	0.795	0.957

TD – training and development; CPR – contingent pay and rewards; PA – performance appraisal; RS – recruitment and selection; CS – competitive salary; ES – employment security; CHSR – community health services results

Statistics related to the structural model analysis are reported in Table 3 and contain values for lower and upper confidence intervals, path coefficients for all relationships and t-values. Statistics of least square approach to structural equation modelling revealed the CS is negatively related to community health service results (CHSR) ($\beta = -0.177$, $p < 0.05$). Study results noted that ES is positively and statistically significant associated with CHSR ($\beta = 0.296$, $p < 0.001$), as well as TD ($\beta = 0.359$, $p < 0.001$). Results showed that CPR, PA, and RS are positively related to CHSR but not statistically significant.

Cross-validated redundancy index (Stone–Geisser Q^2) has been calculated for CHSR as endogenous latent variable. The value of the coefficient Q^2 was recorded as 0.570 for the independent variable and this result demonstrated an excellent score. For the same construct, the coefficient of determination explained variance (R^2) was calculated to assess the model's explanatory power. The R^2 value was 0.592 and revealed strong model's explanatory power indicating that the model provides an explanation for more than 50% of the variance. Standardized root mean square residual for the model was 0.059 and is significantly below the 0.08 criterion [26]. Goodness-of-fit is recorded within acceptable range.

DISCUSSION

The results of our study are consistent with the evidence obtained in previous research, but at the same time they bring new insight into the relationship between HRM practices and CHSR. In general, the results of the statistical analysis confirmed that HRM is an important factor for the operational capability of the healthcare system [1]. HR practice related to competitive salary has a significant negative impact on CHSR. This result reveals that current earnings in public health organizations do not meet the criterion of external competitiveness. Indirectly, this mismatch affects the motivational mechanism and weakens the potential for providing quality patient care. ES records a strong positive association with health service results and gives an indication for an indirect conclusion that

Table 3. Results of testing direct effects

Relationship	Path coefficient	t-value	95% CI (bias-corrected)	Results
CS → CHSR	-0.177*	2.132	[-0.335, -0.014]	Supported
CPR → CHSR	0.152	1.829	[-0.009, 0.315]	Not supported
ES → CHSR	0.296***	4.027	[0.157, 0.442]	Supported
PA → CHSR	0.130	1.269	[-0.076, 0.325]	Not supported
RS → CHSR	0.074	0.693	[-0.142, 0.277]	Not supported
TD → CHSR	0.359***	4.205	[0.193, 0.531]	Supported
Construct	Stoner–Geisser Q2	R2	GOF	
CHSR	0.570	0.592	0.581	
SRMR	0.059			

TD – training and development; CPR – contingent pay and rewards; PA – performance appraisal; RS – recruitment and selection; CS – competitive salary; ES – employment security; CHSR – community health services results; GOF – goodness-of-fit; SRMR – standardized root mean square residual
*p < 0.05;
**p < 0.01;
***p < 0.001

healthcare organizations with a lower turnover rate achieve good health service results [21]. HR practice related to TD achieves the strongest relationship with health service results. This unequivocally confirms that a wide range of opportunities for learning and competence development of employees in public healthcare organizations contributes to the quality of health services. This relation is indirect rather than direct and can be realized through the activation of intrinsic motivation which can have a positive effect on job satisfaction [27] and create the potential for better performance of healthcare professionals. Summarizing the above, it can be concluded that the practice and policy of HRM can play a significant role in increasing the quality of health service results of public healthcare organizations, both through improving patient care and quality of life [20, 28], and increasing client satisfaction [12]. Despite previous research that indicated the importance of managing the performance of employees in healthcare organizations, the results of our study did not reveal the association between performance appraisal and health service results [7]. An important result of the study is the absence of effects of HR practices related to CPR, and staffing policy. This provides an incentive for new research and focusing attention on certain elements of HR practice.

The analysis of the individual relations included in the research model of our study provides a clear theoretical contribution. Confirmation of a statistically significant relationship between TD activities of HRM and CHSR is in accordance with human capital theory [29]. Investments in human capital contribute to the creation of potential for increasing productivity and organization's value towards improving knowledge and competence. The results of the study also greatly support the key postulates of the resource-based theory of the firm [30]. Healthcare professionals employed in public healthcare organizations have been identified as an important factor that affects the performance of healthcare organizations and contributes to their sustainability.

CONCLUSION

The analysis of the relationship between HRM practice and community health services outcome showed that HR practices can be effective in contribution to the improvement of the quality of service of public health organizations. Based on the obtained results of the study, it is possible to identify theoretical implications with a clear practical purpose. The conclusion is that not all HR practices implemented in public healthcare organizations have the same effect on CHSR. The proven effectiveness of TD as well as ES shows that positive changes in CHSR can be expected by focusing on this HR practice. This result can also be achieved through HR practices related to competitive salary, with prior fulfillment of external equivalence conditions. Further research will be aimed at increasing the sample through the participation of healthcare professionals who are employed in private healthcare organizations. This would create assumptions for multigroup analysis, comparison of HR practices in private and public healthcare organizations and conclusions about their effectiveness.

Conflict of interest: None declared.

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Управљање људским ресурсима и исход здравствених услуга – разоткривање односа у јавним здравственим организацијама

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САЖЕТАК

Увод/Циљ Управљање људским ресурсима и повезане праксе представљају широко истраживачко подручје у оквиру здравствених организација. Прилагођавање пракси управљања људским ресурсима текућим условима на тржишту рада и постизање организационих циљева у домену задовољавајућег квалитета здравствене услуге представљају значајне текуће изазове и стварају изазов за истраживање. Циљ студије је да испита однос између праксе управљања људским ресурсима и исхода здравствених услуга здравствених организација у јавном сектору.

Методе Истраживање је конципирано као студија пресека. За прикупљање примарних података коришћен је структурирани упитник. Узорак чини 257 здравствених радника запослених у здравственим организацијама у јавном сектору. За процену статистичке значајности односа у истраживачком моделу примењен је метод структурних једначина.

Резултати Наша студија је показала да је пракса конкурентних зарада негативно повезана са исходом здравствених услуга (β = -0,177, p < 0,05) здравствених организација у јавном сектору. Резултати студије су показали да је сигурност запослења позитивно повезана са исходом здравствених услуга (β = 0,296, p < 0,001), као и са обуком и развојем запослених (β = 0,359, p < 0,001).

Закључак Резултати студије имају теоријске импликације кроз допринос постојећој теорији људског капитала и теорији фирме заснованој на ресурсима. Идентификација праксе управљања људским ресурсима која позитивно доприноси исходу здравствених услуга даје јасне практичне импликације за менаџере здравствених организација у јавном сектору.

Кључне речи: управљање људским ресурсима; пракса управљања људским ресурсима; исход здравствених услуга; организационе перформансе

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Adult-onset Still's disease and Muckle–Wells syndrome – two sides of the same coin?

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SUMMARY

Introduction Adult-onset Still's disease (AOSD), a systemic inflammatory disorder, often represents a heterogeneous entity and diagnosis requires the exclusion of mimicking disorders, including autoinflammatory diseases. We present a patient who meets the diagnostic criteria for AOSD and Muckle–Wells syndrome (MWS).

Case outline A 35-year-old male presented with lymphadenopathy and a chronic nonspecific rash, fever spikes, widespread arthralgia, and joint effusions. Laboratory results showed increased inflammation, leukocytosis, neutrophilia, thrombocytosis, and elevated liver enzymes, accompanied by negative immunoserological tests. Patient was diagnosed with AOSD and prednisone (15 mg/d), methotrexate (10 mg/w) and chloroquine (250 mg/d) are introduced in therapy. Due to refractory course, patient was introduced with anti IL-6 biological agent tocilizumab in 2014 (8 mg/kg monthly). However, after three doses, the drug is stopped due to disease exacerbation. In 2015, there was suspicion that there was another underlying disease from the autoinflammatory spectrum, but DNA analysis of the most common mutations in the NLRP3 gene was negative. In 2017, an ear, nose, and throat specialist confirmed bilateral sensorineural hearing loss, and in 2019, amyloidosis was confirmed after biopsy of the duodenum. Patient fulfilled a new-proposed diagnostic criteria for MWS and confirmation of mutation in *NLRP3* gene is not obligatory according to Eurofever registry.

Conclusion The symptoms of AOSD and MWS partly overlap, as well as their diagnostic criteria. In chronic refractory cases of AOSD, evaluation of diagnosis should be performed and autoinflammatory syndromes must be kept in mind.

Keywords: adult-onset Still's disease; Muckle–Wells syndrome; autoinflammatory diseases

INTRODUCTION

Adult-onset Still's disease (AOSD), a rare systemic inflammatory disorder, is often considered a part of the spectrum of the better-known systemic-onset juvenile idiopathic arthritis, with later age onset. The diagnosis is primarily clinical and necessitates the exclusion of a wide range of mimicking disorders. AOSD is a heterogeneous entity, usually presenting with high fever, arthralgia, typical salmon-pink skin rash, lymphadenopathy, and hepatosplenomegaly accompanied by systemic manifestations and hyperferritinemia. Clinical presentation is exceedingly variable and the disease has no clinical, biochemical, or radiological biomarker. The diagnosis is based on clinical and empirical evidence, with patients meeting inclusion and exclusion criteria and having negative immunoserological results [1, 2].

Autoinflammatory diseases are increasingly recognized and are in the differential diagnosis of many disease states [3]. Cryopyrin-associated periodic fever syndrome (CAPS) is a group of monogenetic diseases consisting of familial cold autoinflammatory syndrome and presenting with urticaria triggered by cold, Muckle–Wells syndrome (MWS) with fever, hearing loss, rash and joint pain, and neonatal onset multisystem inflammatory disease, a severe neonatal disease. This CAPS entity represents a third most common autoinflammatory disease, besides familial Mediterranean fever and tumor necrosis factor receptor-associated periodic syndrome [4]. CAPS is associated with mutations in the *NLRP3* gene (Nod-like receptor Family Pyrin Domain Containing 3) which encodes for protein cryopyrin. Cryopyrin associates with the apoptosis-associated speck like protein and pro-caspase 1 to form the *NLRP3* inflammasome, which is important for activation of pro–interleukin 1β (IL-1β) to mature IL-1β [5]. Consequently, CAPS can be treated with anti-IL-1β therapy [6]. Some patients with more diffuse inflammatory symptoms together with *NLRP3* mutations have been classified as "atypical" CAPS.

According to recently published papers, AOSD and CAPS (especially MWS) share a few similarities in pathogenesis. That explains a similar epidemiology, clinical presentations of both conditions which is difficult to differentiate and good response to anti IL-1 treatment [6, 7]. We describe a patient with inflammatory

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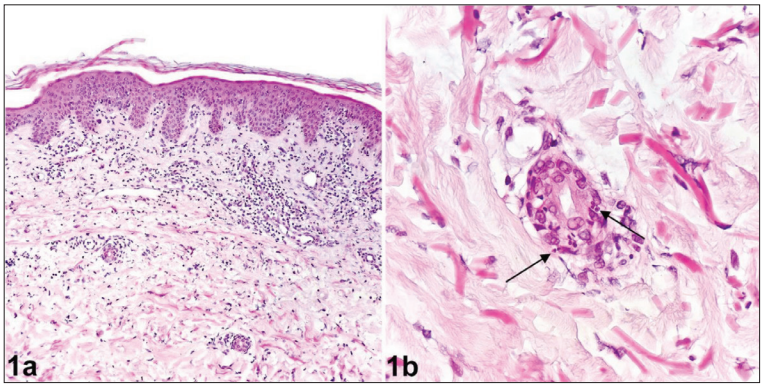

Figure 1. Mixed inflammatory infiltrate composed of neutrophils, eosinophils, and lymphocytes is present in the papillary and reticular dermis (1a); at high magnification, the presence of neutrophils within the sweat gland ducts (epitheliotropism) can be observed (arrow) (1b)

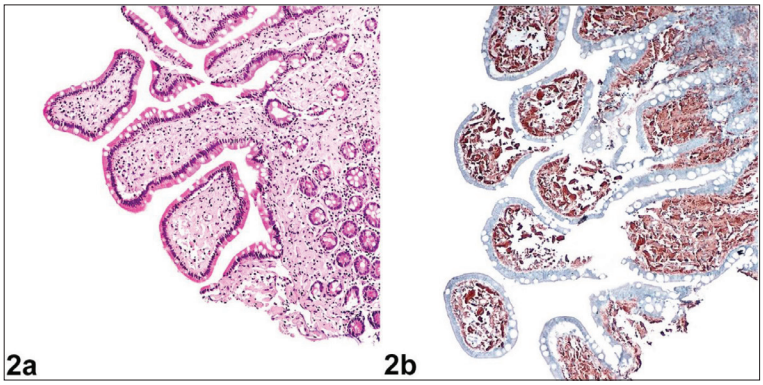

Figure 2. Eosinophilic deposits of amyloid are observed in the lamina propria of the duodenum (2a); immunohistochemical staining for amyloid A shows positivity (2b)

symptoms fulfilling a few proposed sets of diagnostic/classification criteria of AOSD but also fulfilling a new proposed diagnostic criterion of MWS.

CASE REPORT

In late 1999, a 35-year-old man presented with chronic nonspecific maculopapular exanthema of the trunk, upper, and lower limbs, accompanied by axillar and inguinal lymphadenopathy. In 2000, the patient was admitted to the Clinic of Allergology and Immunology of the Clinical Centre of Serbia. Laboratory results showed increased markers of inflammation [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)], leukocytosis, thrombocytosis and elevated hepatic enzymes [aspartate transferase (AST), alanine transferase (ALT), gamma-glutamyl transferase]. After exclusion of hematological malignancies (lymph node and bone marrow biopsy was performed on two occasions) and infections (virus serologies, hemocultures, stool, and urine cultures were negative) patient was diagnosed with idiopathic chronic urticaria and reactive lymphadenopathy. In 2003, the patient was hospitalized twice during a short period: first at the Clinic of Dermatovenereology and then at the Institute of Rheumatology due to lymphadenopathy and chronic non pruritic urticaria resistant to treatment. Urticarial rash was associated with fever spikes (< 38°C), widespread arthralgia and left knee effusion. Analysis of the left knee aspirate showed nonspecific inflammatory synovial fluid. Laboratory results showed increased inflammation (ESR 48 mm/h, CRP 80 mg/l), leukocytosis (19.1 × 10^9/L), neutrophilia (12.5 × 10^9/L), thrombocytosis (654 × 10^9/L), anemia of chronic disease and elevated enzymes, slight above upper reference limit (AST, ALT, lactate dehydrogenase), with negative immuno-serological tests: rheumatoid factor, anti-citrullinated protein antibodies, anti-double stranded DNA antibody, anti-Smith antibody, anti-SSA/Ro autoantibodies, anti-SSB/La autoantibodies, antineutrophil cytoplasmic antibodies, and cryoglobulins. Ultrasound of the abdomen revealed slight hepatosplenomegaly, while conventional radiography of the knees, hands, and feet excluded chronic erosive arthritis. A specialist of infectious diseases and a hematologist were again consulted for exclusion of these diseases (most common tumor markers were negative). According to patient history, clinical findings and course of the disease, imaging techniques results and laboratory findings patient is diagnosed with AOSD; diagnosis is based on presence of lymphadenopathy, slight hepatosplenomegaly, leukocytosis, neutrophilia and thrombocytosis, elevated hepatic enzymes, non-erosive polyarthritis, fever, and atypical urticarial rash. The patient met the proposed diagnostic and classification criteria for AOSD, as outlined by Cush et al. [8], Fautrel et al. [9], and Calabro, primarily using the Yamaguchi criteria. Prednisone (15 mg/d), methotrexate (10 mg/w) and chloroquine (250 mg/d) are introduced in the therapy. From 2003 to 2011 the patient was hospitalized at the Institute of Rheumatology several times due to persistent low-grade fever, articular and skin symptomatology, which were resistant to treatment (instead of chloroquine leflunomide was introduced, dosage of prednisone and methotrexate was increased with wide symptomatic therapy). According to literature, the first line treatment for chronic refractory (dominantly articular) of AOSD is IL-6 inhibitors and tocilizumab is proposed for further therapy [10]. Biological agent was introduced to therapy in 2014 (8 mg/kg monthly) and after three doses of tocilizumab, the drug was stopped due to disease exacerbation. Laboratory findings indicated intense increase in inflammatory markers, with marked leukocytosis, neutrophilia and thrombocytosis. Due to the exacerbation of the skin changes, skin biopsy was performed, and results showed urticaria with the mixed lymphocyte/neutrophil infiltration (Figure 1A and 1B).

At that time, the patient reported a bilateral hearing impairment which was a new symptom in clinical course of the disease, accompanied by persistent slight proteinuria (< 300 mg). All immune-serological tests for vasculitis and other systemic rheumatological conditions were negative

again. In 2015, for the first time, there was suspicion about another underlying disease, and blood sample for DNA analysis is sent to a foreign laboratory for testing for the most common mutations in *NLRP3* gene, but the tests was negative. In 2016, the patient was prescribed with anti-gout agent colchicine 0.6 mg daily with dosage increase up to 1.8 g daily in 2018, with poor medication compliance. Regarding the clinical course of the disease, in 2017 an ear, nose, and throat specialist confirmed bilateral sensorineural hearing loss after severe hearing impairment. Serum amyloid A (SAA) was highly increased in the sera of the patient in April 2019 (968 mg/L, cut off 6.4), biopsy of abdominal fat pad was inconclusive due to severe weight loss, but amyloidosis is definitely confirmed after biopsy of duodenum in 2019 due to persistent gastrointestinal bleeding (Figure 2A and 2B).

These three manifestations – urticaria, hearing loss, and amyloidosis – were first described as *UDA syndrome* in 1962 by Muckle and Wells [11]. In July 2019, due to the chronic course of the disease and no response to previous therapy, there was a request from a rheumatologist to the Republic Expert Committee for Rare Diseases for anti IL-1 drug treatment (anakinra or canakinumab). However, this request was not approved, so patient continued to take 10 mg of prednisone daily and 15 mg of methotrexate, and this was the very last data from patient's medical record.

All procedures were carried out in compliance with the institutional and/or national research committees' ethical standards, as well as the 1964 Helsinki Declaration and its revisions or similar ethical standards. The patient provided written permission to publish all shown material.

DISCUSSION

The patient fulfilled a newly proposed diagnostic criteria for MWS and confirmation of mutation in *NLRP3* gene is not obligatory according to Eurofever registry [12, 13]. To the best of our knowledge, and after extensive literature search, only two papers are published about association of AOSD and MWS in the past ten years [14, 15], but the diagnosis was still uncleared in our patient only. However, authors of recently published papers report new mutation in *NLRP3* gene, thus, directing all of us to look for new mutations and variants [16, 17]. Both MWS and AOSD are characterized by urticaria which exhibit specific histological characteristics that distinguish them from urticaria not associated with systemic diseases. Specifically, urticaria seen in MWS and AOSD is characterized by the presence of epitheliotropism (neutrophils within epithelial structures of the epidermis or eccrine sweat glands) [18]. The presence of epitheliotropism in skin biopsies of patients with urticaria should raise suspicion and warrant further investigations for underlying autoinflammatory conditions.

Both, MWS and AOSD, can present with secondary amyloid A (AA) amyloidosis as a complication [19]. It is characterized by the extracellular deposition of fibrils composed of fragments of the SAA protein; a major acute-phase reactant protein primarily produced by hepatocytes. AA can be detected through histochemical staining with Congo red or immunohistochemical staining for AA (Figure 2A and 2B).

The symptoms of AOSD and CAPS, especially MWS, overlap, as well as diagnostic criteria, so different sets of criteria may be challenging. Our patient fulfilled the Yamaguchi (and other proposed) criteria, responded partially to higher doses of steroids and conventional synthetic disease-modifying antirheumatic drugs, with lymphadenopathy, hepatosplenomegaly and elevated liver enzymes supporting AOSD as primary diagnosis. Bilateral sensorineural hearing loss is suggestive for phenotypic CAPS/MWS. Other characteristics such as arthralgia/arthritis, leukocytosis, thrombocytosis, neutrophilia and anemia can be seen in both conditions. Regarding the nonspecific urticarial rash as well as confirmed amyloidosis, they also can be seen in both conditions.

Unfortunately, the patient died at home, in late 2019; an autopsy was not performed, so a possible cause of death could be macrophage activation syndrome [20], the most severe complication, gastrointestinal bleeding, or a cardiovascular event.

The treatment of choice for both conditions, refractory AOSD and MWS, is IL-1 inhibition (anakinra or canakinumab), with canakinumab showing long-term efficacy and safety [21]. In September 2022, the first anti IL-1 drug (canakinumab) was registered in Serbia for pediatric monogenic autoinflammatory diseases as well as refractory AOSD. In chronic refractory cases of AOSD, evaluation of diagnosis should be performed, and autoinflammatory syndromes must be kept in mind in differential diagnosis because both diseases are highly heterogeneous and share wide similarities.

Conflict of interest: None declared.

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Стилова болест одраслих и Макл–Велсов синдром – две стране исте медаље?

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САЖЕТАК

Увод Стилова болест одраслих је системско запаљенско обољење код којег постављање дијагнозе захтева претходно искључивање сличних обољења као што су аутозапаљенске болести. Приказаћемо болесника са системском запаљенском болешћу који истовремено испуњава дијагностичке критеријуме за Стилову болест одраслих и Макл–Велсов синдром.

Приказ болесника Код 35-годишњег мушкарца се манифестовала лимфаденопатија са хроничним неспецифичним кожним променама, скоковима повишене телесне температуре, боловима и отоцима зглобова. У лабораторијским анализама су виђени повишени маркери запаљења, леукоцитоза, неутрофилија, тромбоцитоза и повишени јетрени ензими, док су сви имуносеролошки тестови били негативни. Постављена је дијагноза Стилове болести одраслих и у терапију су укључени пронисон® (15 *mg/d*), метотрексат (10 *mg/n*) и хлороквин (250 *mg/d*). Због рефракторног тока болести, 2014. године је укључен биолошки анти *IL*-6 лек тоцилизумаб (8 *mg/kg* месечно), али је лек обустављен после три дозе због погоршања болести. Током 2015. године постављена је сумња на друго обољење из спектра аутозапаљенских болести, али је ДНК анализа најчешћих мутација у гену *NLRP3* била негативна. Специјалиста ОРЛ је 2017. године потврдио дијагнозу обостраног сензоринеуралног оштећења слуха, а 2019. године је потврђена и дијагноза амилоидозе на основу биопсије дуоденума. На основу нових симптома, болесник је испунио новопредложене дијагностичке критеријуме за Макл–Велсов синдром и потврда мутације у *NLRP3* гену није била неопходна за дијагнозу.

Закључак Симптоми Стилове болести одраслих и Макл–Велсовог синдрома делимично се преклапају, као и њихови дијагностички критеријуми. Потребна је реевалуација дијагнозе Стилове болести код свих болесника са рефракторним током болести, са освртом на аутозапаљенске болести.

Кључне речи: Стилова болест одраслих; синдром Макл–Велс; аутозапаљенске болести

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Full-thickness chest wall reconstruction after resection of recurrent desmoid-type fibromatosis

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SUMMARY

Introduction Desmoid-type fibromatosis (DF) is a benign but locally infiltrative soft tissue tumor that develops from fascia and musculoaponeurotic tissue with high local recurrence rate. The aim of this article was to present a case of recurrent DF of the chest wall and chest wall reconstruction after tumor resection.

Case outline A 62-year-old man came for an examination due to a tumor localized on the right anterior chest wall. The previous year, the patient had undergone surgical excision of DF of the abdominal wall. Physical examination found a firm and painless 6–7 cm mass on the right side of the chest wall with no swelling of superficial lymph nodes. A computed tomography scan revealed a homogenous mass of soft tissue density, measuring 7.12 × 4.23 cm, arising from right anterolateral wall of thoracic cage with adjoining ribs destruction. The patient was taken to surgery, and right thoracotomy was done with excision of tumor along with resection of the eighth, nineth and tenth rib. The results of pathological examination were consistent with the frozen section, and the patient was diagnosed with DF. Despite the absence of postoperative radiotherapy, there was no evidence of local recurrence two years later.

Conclusion Surgical treatment of recurrent DF of the chest wall requires a wide resection with negative margins. Multidisciplinary approach in case of full-thickness defect of the chest wall and combination of pedicled muscle flap and polypropylene mesh are important to provide chest wall stability.

Keywords: fibromatosis; chest wall resection; surgery

INTRODUCTION

Desmoid-type fibromatosis (DF) is a benign but locally infiltrative soft tissue tumor that develops from fascia and musculoaponeurotic tissue [1]. DF was first described in the abdominal wall and later in the extremities and chest wall with high incidence of local recurrence [1, 2]. Recurrent DF displayed a different biological behavior then primary disease and excision of recurrent DF is associated with high risk of further recurrence [2]. Treatment of recurrent DF with infiltration of ribs is challenging and requires a multidisciplinary approach [3].

We present a case of full-thickness chest wall reconstruction with a pedicled latissimus muscle flap (LD) and polypropylene mesh after the resection of recurrent DF.

CASE REPORT

A 62-year-old man presented with a two-months history of a right chest wall mass. The patient had undergone surgical excision of DF of the abdominal wall the previous year. Physical examination found a firm and painless 6–7 cm mass on the right side of the chest wall attached to the deep tissues with no swelling of superficial lymph nodes (Figure 1a). A computed tomography scan revealed a homogenous mass of soft tissue density, measuring 7.12 × 4.23 cm, arising from the right anterolateral wall of the thoracic cage with adjoining ribs destruction (Figure 2). The patient was taken to surgery, and right thoracotomy was done. Wide resection with a tumor free margin (> 3 cm) along with the resection of adjacent muscles of chest and abdominal wall and the eighth, nineth, and tenth rib were performed (Figure 1b and 1c). The reinsertion of diaphragm and reconstruction of the abdominal wall with polypropylene mesh were performed as well (Figure 1d and 1e). The right LD was harvested and transposed through subcutaneous tunnel to the full-thickness chest wall defect (Figure 1f and 1g). Primary closure of wounds using four Redon drains was performed (Figure 1h). The patient was transferred to the intensive care unit and moved to general care after 24 hours. He was discharged from the hospital after eight days. The sutures were removed on day 14 after the surgery. The results of pathological examination were consistent with the frozen section, and the patient was diagnosed with DF. Despite the absence of postoperative radiotherapy, there was no evidence of local recurrence two years later (Figure 1i).

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and

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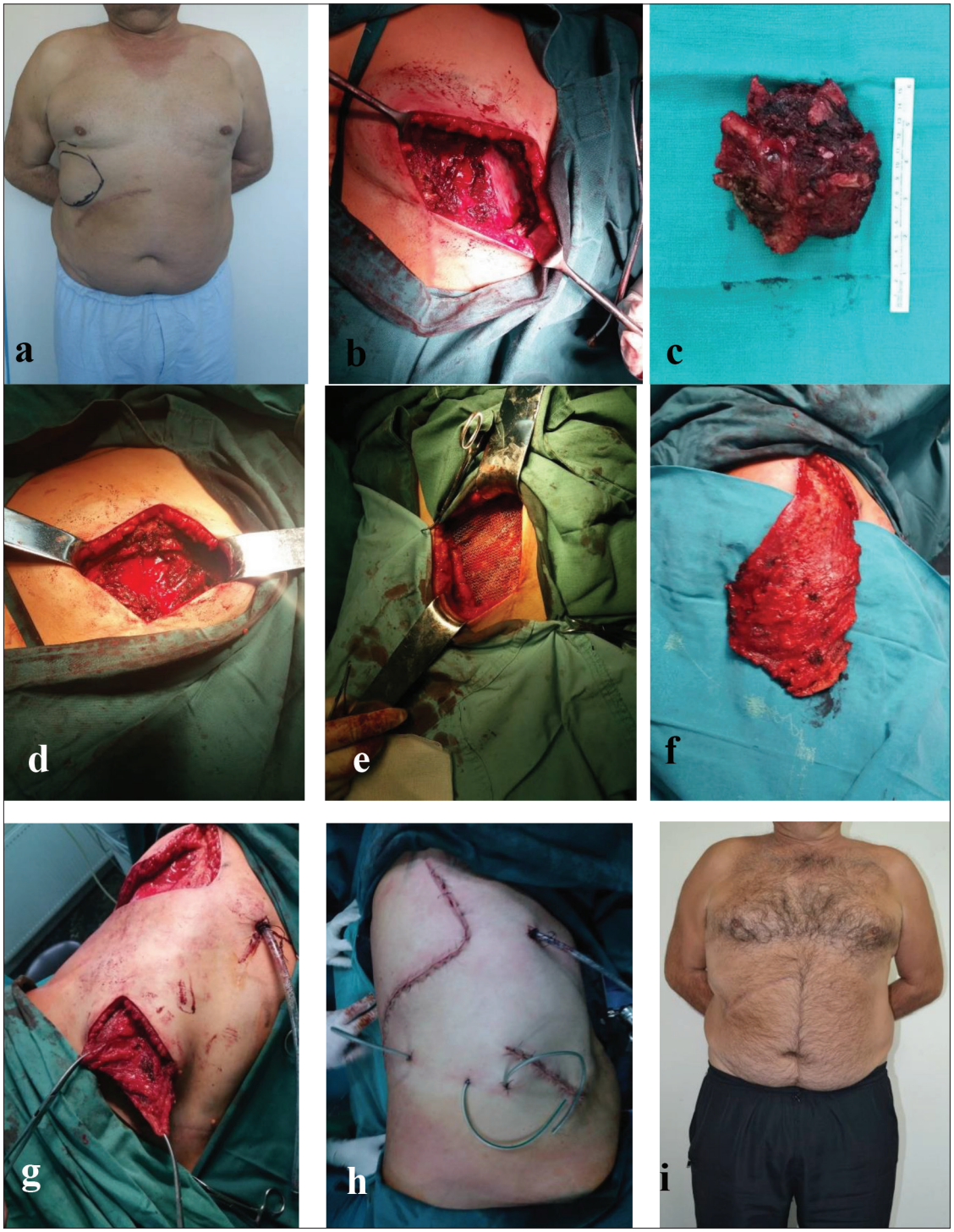

Figure 1. a – Patient presenting a desmoid-type fibromatosis on the right anterolateral chest wall; b – full-thickness defect after resection of desmoid-type fibromatosis; c – surgical specimen showing the tumor with adjacent ribs segment; d – reinsertion of diaphragm; e, f, g, and h – subsequent reconstruction of full-thickness chest wall defect and abdominal wall defect with polypropylene mesh and pedicled latissimus dorsi muscle flap; i – postoperative result after two years

with the Helsinki Declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Chest wall tumors have broad potential etiologies and surgery is considered a potentially curative treatment [4]. Around 20% of DF are located in the chest wall. DF has a high local recurrence rate, and the goal of surgery is achieving negative margins without causing critical functional defects. The positive margins after resection are associated with high incidence of local recurrence [5, 6]. Aggressive and extensive resection are very important in successful management of DF. In the case when DF invade vital structures, obtaining negative margins often represents a therapeutic challenge for thoracic and

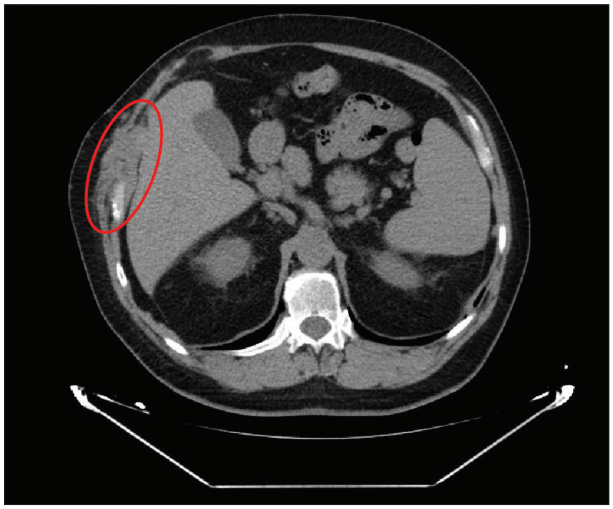

Figure 2. A computed tomography scan of the chest: tumor of the right anterolateral chest wall, dense tissue tumor arising from the anterolateral chest wall

reconstructive surgeons [4, 7]. Other treatment options for DF include radiation therapy, chemotherapy, antihormone agents, and tyrosine kinase inhibitors [1, 5]. Radiotherapy is recommended for patients with positive margins or unresectable tumor [8]. Treatment of recurrent DF of the chest wall remains a challenge due to infiltration of muscles and bone structure [1, 9].

In this case, we performed full-thickness anterolateral chest wall reconstruction with polypropylene mesh and LD after radical excision of DF. The DF invaded the eighth, nineth, and 10th rib with adjacent muscles. The full-thickness chest wall reconstruction must provide chest wall stability and avoid paradoxical motion [5, 10].

The chest wall defects after resection of four or less consecutive ribs can be stabilized with Gore-Tex patch or polypropylene mesh with providing a good soft tissue cover for alloplastic materials [6, 7]. The alloplastic materials are to be used in chest wall reconstruction, but they carry the risk of infection. In case of infection, the alloplastic material must be removed [3, 8]. Wide range of the muscle or musculocutaneous flaps can be used to cover alloplastic materials and support chest wall stability such as LD, rectus abdominis flap, pectoralis major flap, omentum flap, or thoracoepigastric flap [11, 12]. The LD is a good choice for chest wall reconstruction in combination with ribs graft or alloplastic materials [12, 13]. The LD flap has minimal donor site morbidity without changes in the shoulder movement. The muscle flap's arch of rotation and effective length may be increased by dividing its humeral insertion. The disadvantages of harvesting the LD are visible donor scar and seroma formation [13].

The treatment of DF of the chest wall is very complicated and requires a multidisciplinary approach. A wide excision with clear resection margins of DF has lower risk of local recurrence. Full thickness defect of the chest wall after wide excision of tumor requires reconstruction with polypropylene mesh and local pedicled muscle or musculocutaneous flaps.

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Conflict of interest: None declared.

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Реконструкција зида грудног коша после одстрањења дезмоидне фиброматозе

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САЖЕТАК

Увод Дезмоидна фиброматоза (ДФ) је бенигни али локално инфилтративни тумор меког ткива који настаје од фасције и мускулоапонеуротичног ткива, са високом стопом локалног рецидива.

Циљ овога рада био је да се прикаже случај рекурентне ДФ зида грудног коша и реконструкција зида грудног коша после одстрањења тумора.

Приказ болесника Приказан је мушкарац старости 62 године, који долази на преглед због туморске промене локализоване на десној страни предњег зида грудног коша. Болесник је подвргнут хируршкој ексцизији ДФ претходне године. Физикалним прегледом утврђена је чврста и безболна маса величине 6–7 *cm* са десне стране зида грудног коша без увећавања површинских лимфних чворова. Компјутеризована томографија је открила хомогену масу густине меког ткива, димензија 7,12 × 4,23 *cm*, која полази из десног предњег спољашњег зида грудног коша са деструкцијом суседних ребара. Болесник је оперисан, урађена је десна торакотомија са одстрањивањем тумора и ресекцијом осмог, деветог и десетог ребра. Резултати патохистолошког прегледа су били у складу са *ex tempore* биопсијом, а болеснику је дијагностикована ДФ. Упркос одсуству радиотерапије, није било локалног рецидива после две године.

Закључак Хируршко лечење рекурентне ДФ зида грудног коша захтева широку ресекцију са негативним рубовима. Мултидисциплинарни приступ у случајевима дефеката пуне дебљине зида грудног коша и комбинација петељкастих мишићних режњева и полипропиленске мрежице важни су за обезбеђивање стабилности зида грудног коша.

Кључне речи: фиброматоза; ресекција зида грудног коша; хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Pathological complete response after primary tumor surgery following chemoimmunotherapy and stereotactic radiosurgery of initially metastatic non-small-cell lung cancer

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SUMMARY

Introduction Surgery of the primary tumor following extended course of chemoimmunotherapy has only recently been recognized as a feasible and safe option for selected groups of patients with initially unresectable non-small cell lung cancer.

Case outline Here we report a case of a 49-year-old female patient, who never smoked, that was diagnosed with metastatic non-small cell lung cancer. Lesions were evident in both lungs and the brain. She underwent stereotactic radiosurgery for brain metastases and combination therapy of chemotherapy, atezolizumab and bevacizumab. Response to therapy was both remarkable and durable. Ten cycles into treatment, magnetic resonance imaging of the brain revealed no metastatic lesions. Positron emission tomography / computed tomography revealed a single lesion in the right upper lobe 22 × 23 mm in diameter. The patient underwent a right upper lobectomy. Pathohistological evaluation of the specimen revealed complete pathologic response. The patient recovered from surgery and continued chemoimmunotherapy. Four months post-surgery she is disease free and of excellent performance status.

Conclusion Primary tumor surgery following extensive chemoimmunotherapy regiment is feasible and could be considered as a treatment option. Further research is warranted to define a patient population that stands to benefit the most from this modality.

Keywords: chemoimmunotherapy; combination therapy; atezolizumab; lung cancer; thoracic surgery

INTRODUCTION

Immune checkpoint inhibitors (ICI) based therapies have become standard of care for the treatment of metastatic non-oncogene addicted non-small cell lung cancer (NSCLC) leading to significant and durable responses in some patients [1]. Recently, surgery of the primary tumor following extended course chemoimmunotherapy therapy has been deemed feasible and safe in selected patients with initial diagnosis of unresectable NSCLC [2, 3]. While there is evidence that local radical therapy is beneficial aid to immune checkpoint inhibitor based systemic therapy in improving clinical outcomes of patients with oligometastatic NSCLC, the role of primary tumor surgery remains undefined [1]. Here we report a case of an oligometastatic NSCLC patient that successfully underwent primary tumor surgery following remarkable response to extended course of chemoimmunotherapy, antiangiogenic agent, and stereotactic radiosurgery (SRS).

CASE REPORT

A previously healthy 49-year-old female patient, who never smoked, presented to the emergency room after a transient loss of consciousness. Magnetic resonance imaging (MRI) of the brain revealed four lesions – two in the left temporal lobe and another two in the left frontal lobe. Further radiographic evaluation of the chest and upper abdomen revealed a spiculated lesion in the right upper lobe 55 × 43 mm in diameter, a subpleural nodule in the left upper lobe 10 mm in diameter. Signs of necrosis were evident in the right hilar mediastinal lymph nodes (Figure 1). Pathohistological evaluation of transbronchial biopsy specimen of the lesion in the left upper lobe confirmed the diagnosis of lung adenocarcinoma. Results of *EGFR* mutations and *ALK* translocations testing came back negative, and the tumor was found to have programmed death ligand1 (PD-L1) tumor proportion score (TPS) of 10%. Given her excellent performance status decision was made to start the treatment with the combination of chemotherapy, atezolizumab, and bevacizumab. The patient also underwent SRS procedure for

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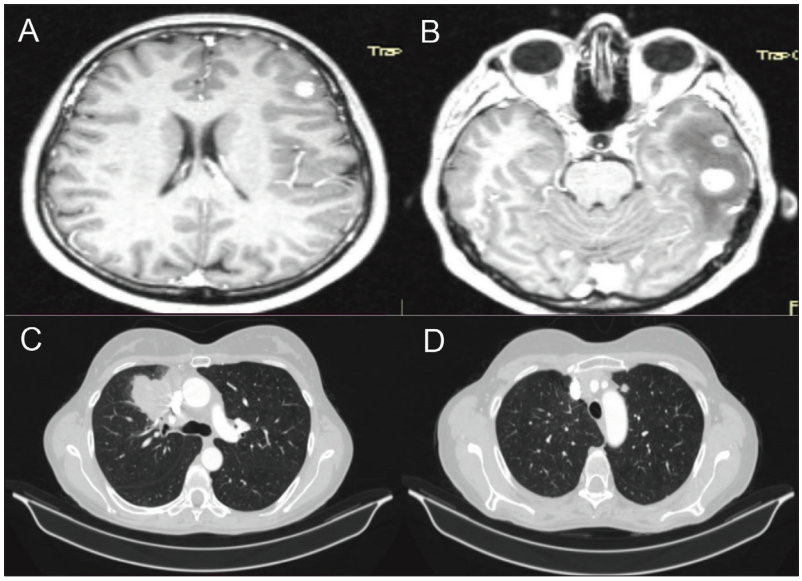

Figure 1. Magnetic resonance imaging of the brain revealing brain metastases in the left frontal and temporal lobes (A, B); multi-slice computer tomography of the chest showing tumor mass in the right upper lobe (C), and a spiculated nodule in the left upper lobe (D) as well as mediastinal lymphadenopathy

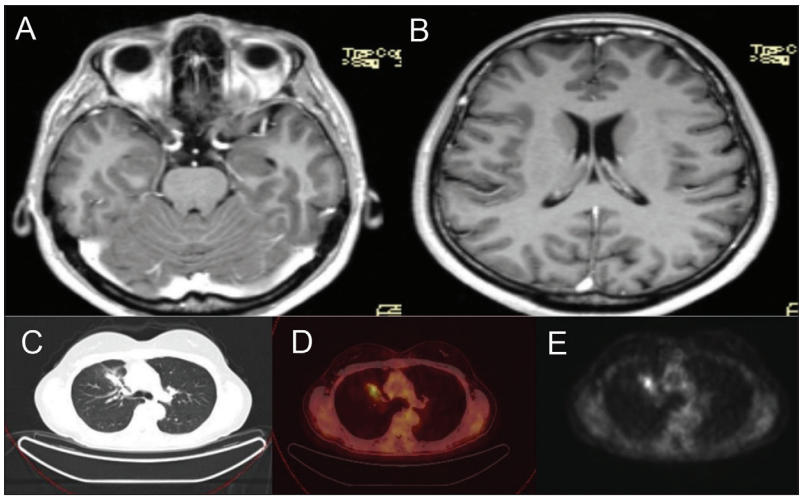

Figure 2. Magnetic resonance imaging of the brain preformed after stereotactic radiosurgery procedure with no detectable lesions (A, B); positron emission tomography / computed tomography following ten cycles of systemic therapy, showing a maintained partial response to therapy and a significant radiopharmaceutical uptake in markedly reduced tumor mass (C, D, E)

the treatment of brain metastases (BM). The radiographic evaluation following the first four cycles revealed partial response per iRECIST criteria with more than 45% reduction in sum of target lesion diameter. MRI of the brain following the SRS procedure showed complete disappearance of the lesions without residual signal changes. Following the eighth cycle of therapy, a further reduction of 17% of sum of target lesions was displayed. Ten cycles into treatment another MRI of the brain revealed no metastatic lesions and PET/CT was performed. (Figure 2) Significant uptake of fludeoxyglucose (SUVmax 4.8) was evidenced only in the lesion in the right upper lobe now 22 × 23 mm in diameter and in the right hilar lymph nodule. The patient underwent a right upper lobectomy with the removal of 12 lymph nodes from the 4R,10R,11R and seventh station ten months after commencing the treatment with chemoimmunotherapy. The postoperative period was uneventful. Pathohistological evaluation of the surgical specimen revealed a pathologic complete response (pCR). She has since continued atezolizumab-bevacizumab therapy and is without tumor reoccurrence four months after surgery.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments and comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Chemoimmunotherapy is now the standard of care for patients with metastatic non-oncogene addicted NSCLC with PD-L1 TPS of 1–49% [1]. With several chemoimmunotherapy regiments recommended, selecting the optimal for a particular patient is a task encountered repeatedly by tumor boards worldwide. Siciliano et al. [4] compared different ICI based treatment strategies for advanced NSCLC in a recently published metanalysis. They found that atezolizumab-bevacizumab-chemotherapy was best ranked in terms of progression free survival (PFS) in non-squamous NSCLC and PD-L1 TPS 1-49% subgroups of patients. Atezolizumab-bevacizumab-chemotherapy ranked best overall for objective response rates [4]. Impressive objective response rates and estimated PFS make this regiment suitable for downstaging of advanced non-squamous NSCLCs as evidenced in our patient's case.

Repots and case series of primary tumor surgeries following downstaging after systemic mono-ICI therapy only recently started surfacing as operating on such patients was deemed risky due to mediastinal and hilar fibrosis that occurs in response to ICI treatment, proving their feasibility in selected group of patients [3, 5, 6, 7]. Recently, reports of patients safely undergoing pulmonary resection following chemoradiotherapy and durvalumab maintenance for initially unresectable NSCLC have been described [6]. This highlights the feasibility of primary tumor surgery even in situations where higher rates of mediastinal and hilar fibrosis are expected.

Chemoimmunotherapy regiments have now found their place in neoadjuvant setting. Results of CheckMate816 trial, evaluating nivolumab-chemotherapy versus chemotherapy alone as neoadjuvant regiment for resectable NSCLC demonstrated significant rates of pCR in the experimental arm (24% *vs*. 2.2%, $p < 0.0001$) highlighting the efficacy of ICIs in this setting [8]. Similar rates of pCR (23.1%)

were observed by Galetta et al. [3] in patients undergoing surgery of primary tumors for previously unresectable NSCLC after tyrosine kinase inhibitor and ICI treatment. After median follow-up of 28.7 months, 82% of patients were alive and the projected five-year survival rate is 66% [3]. In another publication, Higuchi et al. [2] reported pCR rate of 30.8% and two year overall survival rate of 76.2% for advanced NSCLC patients that received mono or chemoimmunotherapy. The median systemic treatment duration prior to surgery of the primary tumor was 10.1 months, similar to our case. None of the patients' treatment regimen however, included an antiangiogenic agent. To the best of our knowledge this is the first case of surgery of the primary tumor following the systemic treatment that includes chemotherapy, atezolizumab and bevacizumab for metastatic NSCLC [2]. Both publications mentioned above included patients with BM at baseline, showing that surgery of primary tumor could be considered following BM treatment. He et al. [9] found that upfront treatment of BM followed by surgery of primary NSCLC was associated with improved survival. Authors concluded that it was the patients that received brain surgery as opposed to radiotherapy for BM treatment derived the most benefit [9]. None of the patients however received ICI in the mentioned study. The abscopal effect has been well recognized, therefore it could be hypothesized that the patients on ICI based therapy stand to benefit more from the addition of BM radiotherapy than those treated with chemotherapy alone [10]. In addition to that, bevacizumab is thought to trigger T-reg cell proliferation and increase T cell infiltration and thus could have contributed to chemoimmunotherapy efficacy in our case [11]. Furthermore, an exploratory analysis of IMpower150 trial found that combination of atezolizumab and bevacizumab could delay the onset of new brain lesions [12]. With all of this in mind decision has been made to continue with the atezolizumab-bevacizumab maintenance therapy as per IMpower150 trial protocol following surgery of the primary tumor [12]. Oligometastatic NSCLC is a vaguely defined condition due to its heterogeneity, but it is thought to be best managed by combining systemic and local radical therapy [1] Surgery of the primary tumor after downstaging is now considered feasible following extended course of chemoimmunotherapy and could provide a good chance for long-term survival for some initially unresectable NSCLC patients. Pathological downstaging and pCR rates after surgery for initially unresectable NSCLC, post-extended chemoimmunotherapy are similar to those in pivotal neoadjuvant trials for resectable NSCLC. pCR has been adopted as an endpoint in neoadjuvant trials for resectable NSCLC and has been associated with favorable clinical outcomes [8]. Its role should be carefully evaluated in patients with initial diagnosis of metastatic NSCLC even after successful downstaging, as it may not reflect complete eradication of the disease thus leaving a gap in knowledge regarding the optimal postoperative treatment and follow-up strategies.

Due to the present widespread use of chemoimmunotherapy and its efficacy, more patients are expected to be eligible for surgery of the primary tumor following downstaging. Further research is warranted to define a patient population that stands to benefit the most from this modality as opposed to multidisciplinary treatment without it.

Conflict of interest: None declared.

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Комплетан патолошки одговор после операције примарног тумора и примене хемоимунотерапије и стереотактичне радиохирургије иницијално метастатског неситноћелијског карцинома плућа

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САЖЕТАК

Увод Хирургија примарног тумора после дуготрајног лечења хемоимунотерапијом недавно је препозната као изводљива и безбедна опција за одабране групе болесника с иницијално нересектабилним неситноћелијским карциномом плућа.

Приказ болесника Представљамо случај 49-годишње болеснице, непушача, код које је дијагностикован метастатски неситноћелијски карцином плућа. Иницијално метастатске лезије биле су присутне у оба плућна крила и мозгу. Мождане метастазе су третиране стереотактичном радиохирургијом, а системско лечење је започето применом хемотерапије, атезолизумабом и бевацизумабом. Одговор на терапију био је изузетно добар и дуготрајан. После десет циклуса терапије, на магнетној резонанци ендокранијума нису детектоване метастатске лезије. Скрининг позитронском емисионом томографијом / компјутеризованом томорафијом открио је само једну лезију у десном горњем режњу, димензија 22 × 23 *mm*. Болесница је подвргнута лобектомији десног горњег режња. Патохистолошка анализа узорка показала је потпуни патолошки одговор. Болесница се опоравила и наставила лечење хемоимунотерапијом. Четири месеца после хируршког захвата, болесница је без детектабилне болести и одличног општег стања.

Закључак Хирургија примарног тумора после продуженог режима хемоимунотерапије изводљива је и може се сматрати опцијом лечења. Потребна су даља истраживања како би се дефинисала популација болесника која би највише имала користи од овог начина лечења.

Кључне речи: хемоимунотерапија; комбинована терапија; атезолизумаб; карцином плућа; торакална хирургија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Pure squamous cell carcinoma of primary pancreatic origin

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SUMMARY
Introduction Primary, "pure" squamous cell carcinoma (SCC) is an exceptionally rare pancreatic malignancy that has been described in sporadic case reports. The appearance of this rare entity created a diagnostic dilemma for us, therefore, in this case report, we are focused on the radiological detection and characterization, pathogenesis, and therapeutic options of pure pancreatic SCC.
Case outline In an 80-year-old female patient, a partially necrotic mass in the tail of the pancreas was detected by computed tomography, which is the rarest localization of this tumor. On the performed imaging, the tumor showed predominantly malignant features with a surprising definitive histopathological diagnosis in the direction of pure SCC. Distal pancreatectomy with splenectomy was performed because of the infiltration of lienal vascular structures.
Conclusion Due to the very aggressive form of this tumor and poor prognosis, early detection, risk factors control, genetic burden, and optimization of surgical and therapeutic management can improve the quality of life and prolong the overall survival period.
Keywords: squamous cell carcinoma; computed tomography; distal pancreatectomy

INTRODUCTION

Primary squamous cell carcinoma (SCC) of the pancreas is a supremely rare entity and the main reason for this is the lack of squamous cells in the pancreas [1, 2]. Pathophysiology is not entirely clear, but there is suspicion of inflammatory-based squamous metaplasia of the ductal columnar cells [3]. There are not many published articles describing the pure SCC of the tail of the pancreas. Consequently, there is no clinical management protocol for this type of pancreatic carcinoma and the survival rate is poor, because of its highly aggressive behavior [2]. In 2020, Qin et al. [4] presented the results of their population-based study that showed for SCC a median overall survival of only three months. Classic risk factors for ductal adenocarcinoma (ADC) like smoking and chronic pancreatitis, do not show association with SCC [5]. Adenosquamous carcinoma (ASC) is another rare entity (with mixed elements of ADC) and squamous carcinoma), but yet more common than pure squamous carcinoma as in our case. ASC is the main differential diagnosis for SCC, but there is also metastatic SCC from other sites in the body and pancreatoblastoma [2, 6].

CASE REPORT

An 80-year-old woman was admitted to the Clinic for Digestive Surgery of the University Clinical Center of Serbia because of epigastric pain and nausea that lasted for a year and a half. Laboratory analyses showed elevated C-reactive protein (11.9 mg/L), as well as elevated tumor markers CA 19-9 (201 kU/L) and carcinoembryonic antigen (CEA) (12.4 μg/L), but the pancreatic amylase (39 U/L) and lipase (52 U/L) were in a normal range. A computed tomography (CT) was performed in our hospital and revealed a necrotic lesion in the pancreatic tail with postcontrast enhanced borders, with a maximum diameter of 52 × 43 mm. The surrounding adipose tissue was of higher density primarily due to the infiltrative growth of the tumor. The contact of the described tumor lesion with the stomach wall and the spleen capsule was clearly visualized (Figures 1 and 2). The splenic vein was thrombosed (Figure 1). Considering the values of CA 19-9 and CEA, the conclusion of the performed diagnosis was a centrally necrotic lesion of the tail of the pancreas with predominantly malignant CT characteristics. An esophagogastroduodenoscopy was performed and even though the extramural compression on the minor gastric curve was

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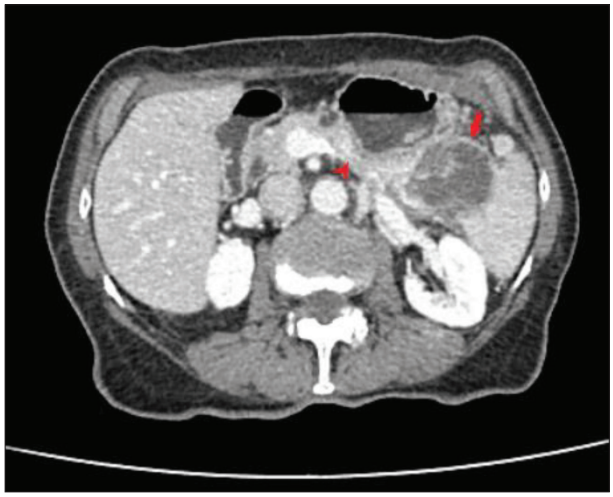

Figure 1. Axial contrast-enhanced computed tomography scan (125 ml iodine contrast, 1 mm slice thickness); the necrotic lesion with post-contrast enhanced borders (arrow) which is in the contact with the gastric body and spleen capsule; thrombosed splenic vein (arrowhead)

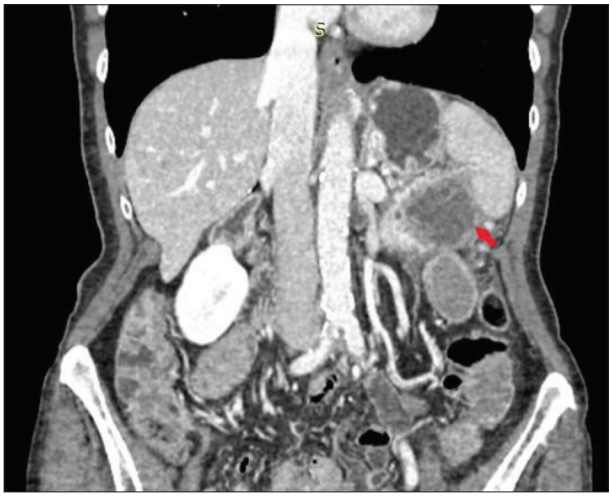

Figure 2. Coronal contrast-enhanced computed tomography scan (125 ml iodine contrast, 1 mm slice thickness); the same necrotic lesion (arrow) which is in this image in contact with the spleen capsule

seen, the mucosa had a normal appearance, without clear signs of infiltration. After preparation, the patient underwent surgery, and distal pancreatectomy and splenectomy were performed. The pathohistological finding was the primary *squamocellulare* invasive carcinoma of the pancreatic tail with a histological aspect of "pure" squamous differentiation of pancreatic carcinoma, showing frequent foci of pseudoglandular arrangement of squamous cells (Figure 3). The patient was discharged from the hospital after 16 days with the decision to receive systemic chemotherapy (5-fluorouracil) in the regional oncological center.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments and comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

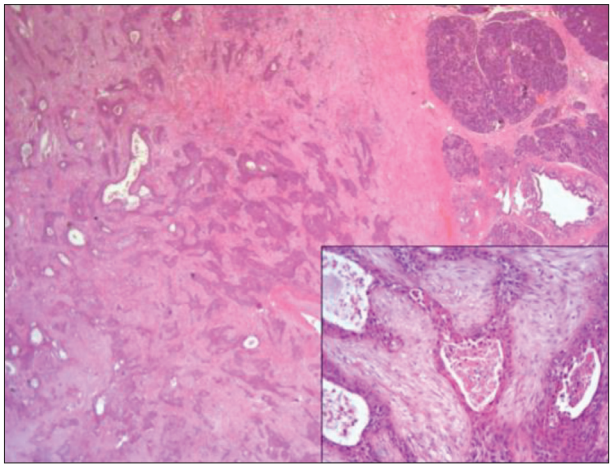

Figure 3. Histological aspect of "pure" squamous differentiation of pancreatic carcinoma, showing frequent foci of pseudoglandular arrangement of squamous cells (inlet picture) suggesting precancerous squamous metaplasia of pancreatic ducts

DISCUSSION

Primary pure SCC of the pancreas is a very rare tumor with an incidence from 0.5% to 2% of all exocrine pancreatic neoplasms [1, 7]. In a survey conducted in Japan back in 1992, researchers investigated 1300 cases of pancreatic cancer through autopsies. Among these cases, only 0.7% were identified as SCC [8]. When comparing pancreatic ADC to pancreatic SCC, the latter is linked with poorer differentiation, displaying a more aggressive nature and leading to worse overall outcomes [9]. Currently, there are no studies available regarding the molecular profile of pancreatic squamous carcinoma. Additionally, there are no retrospective or prospective studies that have identified the optimal therapy for these tumors. Unlike pancreatic ADC, risk factors like smoking and chronic pancreatitis do not seem to be associated with pancreatic SCC.

Due to its rarity, sporadic case reports of this exceptionally unusual tumor have been published. The appearance of this rare entity created a diagnostic dilemma for us and required a review of the available literature, therefore, in this case report we are focused on the radiological detection and characterization, pathogenesis, and therapeutic options of pure pancreatic SCC [10]. Exocrine pancreatic lesions such as SCC and ADC arise from the ductal tissue. A form of ASC has also been described. The origin of this tumor is based on squamous metaplasia of ductules along with other pathogenic factors that contribute to malignant alteration [10, 11]. Some of the theories explaining malignant differentiation to SCC mention the neo-transformation of multipotent stem cells as well as aberrant or ectopic squamous cells. Differentiation of ADC into SCC is also possible, as well as a mixed tumor form [12]. There is a frequent association of this malignancy with chronic pancreatitis, which is considered a risk factor. There is not much data on gene mutations associated with SCC, but there are indications of a hereditary influence as well as the detection of *BRCA-2* mutation in a few published cases [10].

In their study, Ford et al. [5] showed that the majority of patients are diagnosed with the condition in their eighth decade of life, and they found no discernible difference in diagnosis based on gender. The leading symptoms include anorexia, pain in the epigastrium and back, and diabetes, depending on the localization in the pancreas, icterus, gastric outlet obstruction, and rarely may occur upper gastrointestinal bleeding [13, 14].

Radiological characterization is very challenging. CT plays a very important role in the evaluation of patients with pancreatic pathology. Likewise, in our case, the CT examination showed a dominantly necrotic lesion with expansive and extra-pancreatic growth. The main differential diagnosis is pancreatic ADC or ASC, in contrast to which SCC shows more pronounced neovascularization, which contributes to CT detection of intralesional postcontrast viable zones [12]. In our case, the peripheral area of the tumor was hyperdense with irregular borders along with a higher density of perilesional fat tissue. The tumor involved the tail of the pancreas, which is the rarest localization when it comes to pure SCC. Vascular invasion is common, as in our patient, where the tumor process infiltrated the lienal vein, so together with the distal pancreatectomy, a splenectomy was performed. The role of positron emission tomography/CT is in the evaluation of the secondary dissemination of the disease as well as in the detection of the possible primary origin of SCC. Unfortunately, at the time of the discovery of this disease, the tumor is often in a locally advanced stage. CT and endoscopic ultrasound-guided needle biopsy or laparoscopic biopsy are procedures that are very important in obtaining an adequate sample of the tumor tissue for further histological and immunohistochemical processing [10]. Palliative chemotherapy and radiotherapy are the only treatment options in unresectable patients. Surgical resection is possible in less than one third of the patients, and bearing in mind the greater aggressiveness of this type of tumor, the prognosis is worse [15]. As Ntanasis-Stathopoulos et al. concluded, survival is significantly longer in patients after surgical resection [13, 16].

Thanks to the progress in prevention in recent years, early detection, and treatment of pancreatic cancer, an improvement in overall survival after resection is observed. Although a very rare and aggressive form of malignant pancreatic process, due to the improvement and optimization of the surgical techniques and neoadjuvant chemoradiotherapy, as well as advances in imaging modalities, we can hope for better results when it comes to the prognosis of the disease [13].

Conflict of interest: None declared.

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„Чисти“ сквамоцелуларни карцином примарно панкреасног порекла

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САЖЕТАК

Увод Примарни, „чисти“ сквамоцелуларни карцином је изузетно редак малигнитет панкреаса, који је описан у спорадичним приказима болесника. Појава овог ретког ентитета створила је дијагностичку дилему за нас, те смо у овом приказу болесника фокусирани на радиолошко откривање и карактеризацију, патогенезу и терапијске опције „чистог“ сквамоцелуларног карцинома панкреаса.

Приказ болесника Код болеснице старе 80 година компјутеризованом томографијом је откривена делимично некротична маса у репу панкреаса, што је најређа локализација овог тумора. На урађеном прегледу, тумор је показао предоминантно малигне карактеристике, са изненађујућом дефинитивном хистопатолошком дијагнозом у правцу „чистог“ сквамоцелуларног карцинома. Урађена је дистална панкреатектомија са спленектомијом, због инфилтрације лијеналних васкуларних структура.

Закључак С обзиром на то да је овај тумор веома агресиван и има лошу прогнозу, рано откривање, контрола фактора ризика, генетског оптерећења и оптимизација хируршког и терапијског лечења могу побољшати квалитет живота и продужити укупан период преживљавања.

Кључне речи: сквамоцелуларни карцином; компјутеризована томографија; дистална панкреатектомија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Clinical and dermoscopic spectrum of age-dependent spitzoid lesions – when to react?

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SUMMARY
Introduction Spitzoid lesions represent a spectrum of melanocytic lesions comprising benign Spitz nevi, intermediate lesions known as atypical Spitz tumors, and Spitzoid melanoma. They tend to be more common in children and young adults, but all age groups can be affected. Due to complexity of their clinical, dermoscopic and histological differentiation, they are extremely difficult to manage, especially in pediatric population.
Outlines of cases In this report, we present a series of six cases with spitzoid lesions in different age groups with different outcomes.
Conclusion With the following case series, we report clinical and dermoscopic features of biologically various spitzoid lesions, appearing in different age groups. We believe that this article will increase knowledge of both physicians and dermatologists about when and how to react when dealing with a patient with spitzoid lesion.
Keywords: spitzoid lesions; Spitz nevus; atypical spitz tumor; spitzoid melanoma; management

INTRODUCTION

Spitzoid lesions present a distinct group of melanocytic lesions with overlapping clinical, dermoscopic and in some cases even histopathological features. They are classified into benign Spitz nevi (SN), intermediate lesions known as atypical Spitz tumors (AST), and spitzoid melanoma (SM) [1]. While typical SN is a pink to reddish-brown or purple-red papule, a highly pigmented variant of SN also exists and is referred to as Reed nevus (RN) [2]. Spitzoid lesions are extremely rare, as only 1–2% of all melanocytic lesions in both children and adults are diagnosed as SN, while AST and SM are even less common than SN [3, 4]. Most lesions are up to 1 cm in diameter, with a wide range of coloration from pink to black [1, 5]. Although more frequently found in childhood and young adulthood, they occur in individuals of all ages. Currently, no consensus guidelines exist for the management of spitzoid lesions, and due to complexity of their clinical, dermoscopic and histological differentiation, they are extremely difficult to manage.

REPORT OF CASES

Case 1

A six-month-old female infant presented to our dermatology department with a pigmented macule in the umbilical region which was discovered by accident during examination. The lesion was dark brown to black, flat, well demarcated, with a diameter of 5–6 mm. Dermoscopy revealed a melanocytic lesion with a starburst pattern typical of a pigmented Spitz nevus (Figure 1). Follow-up was suggested, every six months, without excision. At the age of two years, the lesion changed symmetrically maintaining the previously noted starburst pattern (Figure 2).

Case 2

A 30-year-old woman presented to our dermatology department with a pigmented lesion on the interior surface of her right upper leg. The patient reported the presence of the lesion since early childhood, but noted that the lesion started changing in color and diameter in the previous several months. Dermoscopic findings revealed an asymmetrical melanocytic lesion, without a clear pattern of pigment distribution. In the center of the lesion milky gray, homogenous pigmentation with subtle pin-point peppering, and remanences of streaks that resemble starburst pattern were found (Figure 3). Wide local surgical excision was suggested and histopathological findings showed features of an atypical spitz tumor, without necessary criteria for the diagnosis of an SM.

Case 3

A 40-year-old woman presented to our dermatology department with a newly acquired pigmented papule on the lateral aspect of her right knee. Dermoscopy showed a melanocytic lesion with homogenic structureless dark pigmentation in the center, surrounded by asymmetrical pseudo-pod-like structures resembling a

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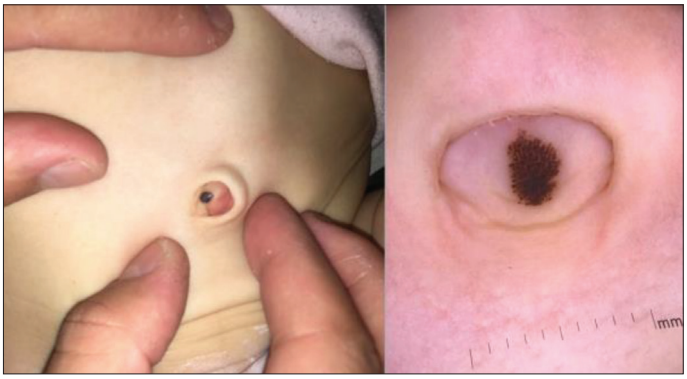

Figure 1. Spitz nevus in the umbilical region of a six-month-old infant

Figure 2. Spitz nevus in the umbilical region of a two-year-old child

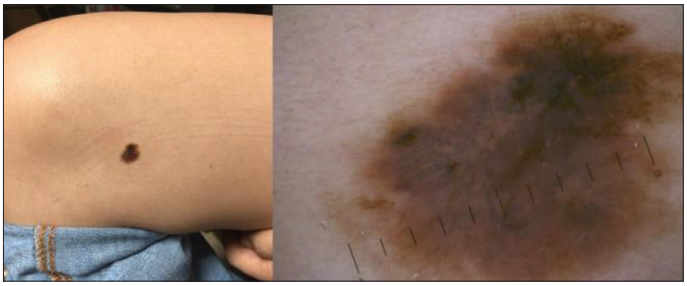

Figure 3. Atypical spitz tumor in a 30-year-old woman

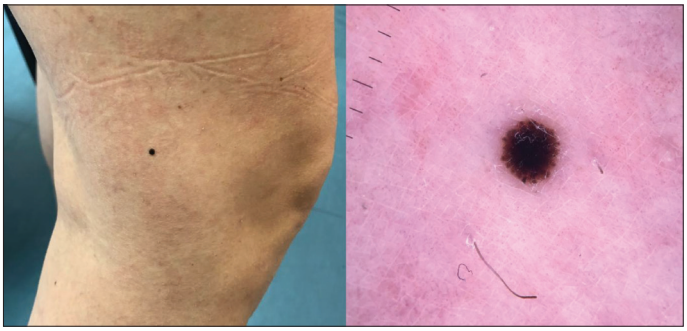

Figure 4. Spitzoid melanoma in situ in a 40-year-old woman

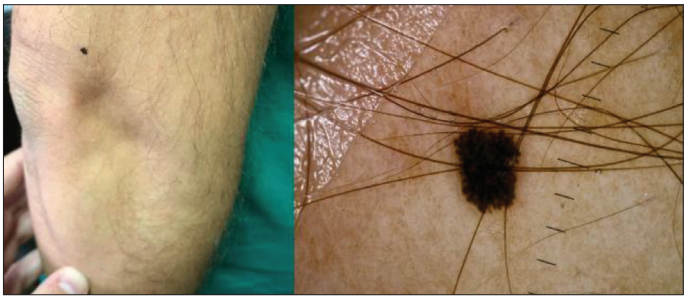

Figure 5. Spitzoid melanoma in a 47-year-old man

starburst-pattern of a Reed nevus (Figure 4). Having in mind the dermoscopic findings and the patient age, wide local surgical excision was suggested. Histopathology revealed atypical epithelioid melanocytes confined to the epidermis and epidermal adnexal structures. The diagnosis of SM *in situ* was made.

Case 4

A 47-year-old man presented to our dermatology department with a newly appeared pigmented papule on his left elbow. Dermoscopy showed asymmetry and radial streaming, pseudopods, and peripheral black dots and globules (Figure 5). Wide local surgical excision was suggested. Histopathology revealed a microinvasive, 0.2 mm-thick SM.

Case 5

A 3-year-old girl presented to our dermatology department with a flesh-colored nodule on the extensor surface of her left lower leg, which had grown over the past six months, more intensively over the past two months. Her mother stated she had the lesion for a longer time, and that it was flat and pigmented prior to the rapid growth phase. Dermoscopy showed a melanocytic lesion, centrally without pigment and with thin interlaced blood vessels. Towards the periphery of the lesion individual globules were present. In one most peripheral zone, the remains of a starburst or coffee bean-like appearance that looked like remnants of a spitzoid lesion (Figure 6). Wide local surgical excision was suggested and histopathology revealed a 2 mm-thick SM, Clark level 4.

Case 6

A 20-year-old man presented to our dermatology department with a pigmented nodular lesion on the extensor surface of his right lower leg. The patient reported a known presence of the observed lesion since his early childhood, also he noted that the lesion started changing in color, diameter and thickness in the past several months Dermoscopy showed remanences of a melanocytic lesion, with absence of color uniformity and pattern symmetry, but also presence of asymmetrically distributed brown dots, pseudopods and central blue–white veil (Figure 7). Wide local surgical excision was suggested and histopathology revealed a 2.3 mm-thick SM, Clark level 4.

All procedures were performed in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patients.

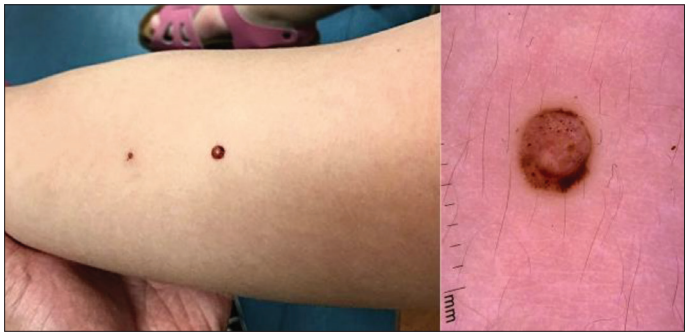

Figure 6. Spitzoid melanoma in a three-year-old girl

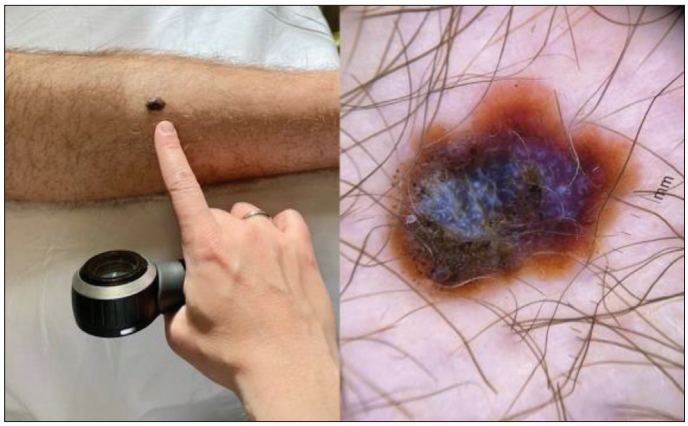

Figure 7. Spitzoid melanoma in a 20-year-old man

DISCUSSION

Spitz nevi present benign spitzoid lesions and are on average less than 6 mm in diameter. They are typically described as dome-shaped, but may as well be flat or polypoid. [3]. A pink to red color is most commonly observed, but flesh-colored and brown to black lesions have also been seen in SN, the latter of which are also referred to as Reed nevi [2, 3]. Some authors consider RN a single nosological entity, but whether SN and RN are distinct lesions, or unpigmented and pigmented variant of the same spectrum is still controversial, due to their both similarities and differences [2].

Atypical spitz tumors are currently defined as tumors of uncertain malignant potential. Their diameter ranges 5–10 mm and they present as a plaque or a nodule in variety of colors ranging from pink to black. The multicomponent pattern is characterized by an uneven distribution of colors and structures [3, 4, 5].

Spitzoid melanomas tend to be larger than AST, with a mean diameter of 1.05 cm, more rarely a SM less than 6 mm can also occur [6]. Spitzoid melanomas are more likely to be nodular, but may also be flat or slightly elevated [3]. Majority of SM lesions have a multi-color pattern as Carrera et al. [7] demonstrated a mean number of 2.7 colors per SM lesion, while the spectrum ranges from red/pink, grey to brown/black.

Dermoscopically, the majority of SN (53%) exhibit a starburst pattern. However, a notable percentage of cases also display globular, coffee-bean like appearance (22%) and atypical pattern (25%) while in hypopigmented SN the most characteristic feature is vascular pattern [2]. Atypical spitz tumors are more challenging to classify as their characteristics overlap with SN and SM [1]. They are more likely to show a non-specific or multicomponent pattern in comparison to SN, but also exhibit a dotted vessel pattern and shiny white lines as frequently as SM [7, 8]. Spitzoid melanomas tend to exhibit more asymmetry and more colors, pink Spitz-like pattern, milky red areas, shiny white lines, red/pink and white coloration, and polymorphous vascular pattern [7].

Age is one of the main criteria to distinguish Spitzoid lesions with indolent behavior from AST with greater risk of malignancy and SM [9]. However, it should not be overly relied on, as although melanomas are rare in pediatric population, SM represent the most common type regarding children, making it the most frequent malignant skin tumor in children [10, 11]. As shown both in the literature and in case of our patient No. 5, nodular spitzoid lesions with multicomponent dermoscopical pattern should always be excised, regardless of age. On the other hand, evolution of SN undergoes from rapid, often dramatic growth phase and in these cases many physicians and even dermatologists tend to raise a red flag, especially when dealing with pediatric population and react advising excision, though unnecessary. These lesions usually stabilize as shown in our case No. 1 and may gradually undergo an involution process [12].

Several studies involving both pediatric and adult patients reported a mean age that ranges from late teens to early 30s, and Lott et al. [14] reported a mean age of 22 years at the time of diagnosis of SN [1, 11, 13]. Very few studies reported an average age of AST diagnosis solely, one of them was conducted by Moscarella et al. [8] and they reported a mean age of 20.8 ± 13.8 years in 55 patients with AST in a multicenter retrospective case–control study. Our patient No. 2 also belongs to this age group, as she was diagnosed at the age of 30 years. The average age for SM diagnoses tends to be higher, with a mean age of 55 years (range 8–90 years) reported by Lott et al. [14] in a 54-cases study. Our adult patients with SM were within this age range as well.

A Study by Bartenstein et al. [6], which included only pediatric patients, demonstrated a median age of 7.4 and 7.2 for SN and AST, respectively, the age group being from three months to 19.7 years, which correlates with the age of our patient No. 1. Carrera et al. [7] reported a mean age of 12.5 years (range 2–20) for SM based on a sample of 15 patients collected in a multicentric retrospective study, as SM is relatively rare in the pediatric population. In the case of our patient No. 5, although by age she was at the lower end of this range, timely excision led to a better prognosis.

In a recent single center 10-year retrospective study comprising 250 spitzoid lesions in pediatric population, conducted by Herzum et al. [15], literature data was confirmed. The results showed that 82% of spitzoid lesions in pediatric patients were benign, though a not negligible

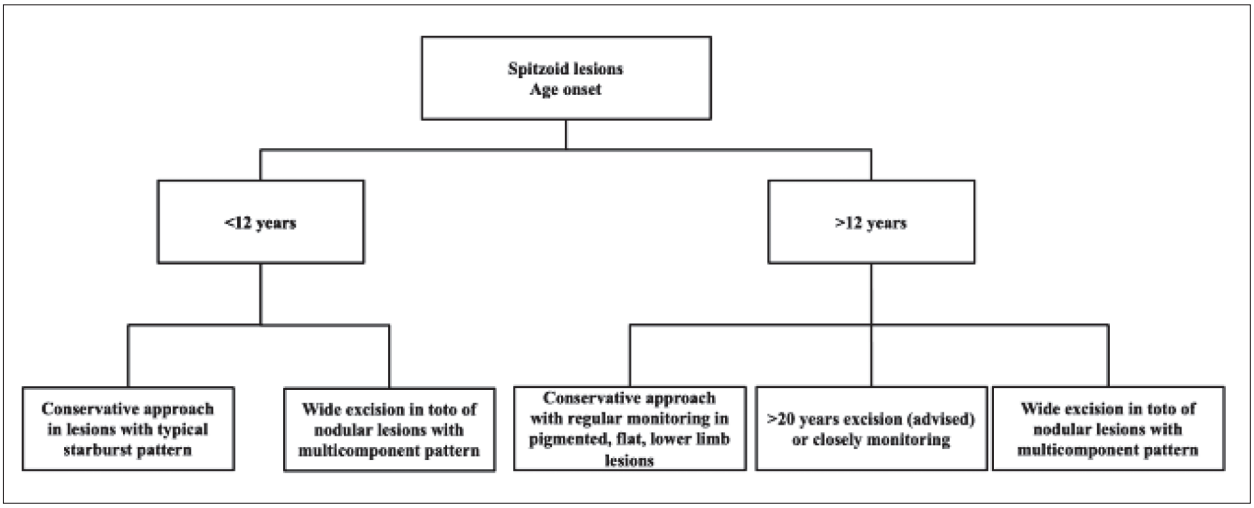

Figure 8. Proposed protocol for the management of spitzoid lesions

percentage of AST (17%) were retrieved, as well as 1% of SM [15]. These findings highlight the need for extreme caution when dealing with spitzoid lesions in childhood, as demonstrated in the case of our patient No. 5.

To date, no specific guidelines or standardized criteria exist for the management of spitzoid lesions, and due to complexity of their clinical, dermoscopic and histological differentiation, they are highly difficult to manage. Numerous guidelines have been developed by several dermatology societies, to help clinicians handle this problem [9, 16, 17]. The majority suggested approach to minimize unnecessary excisions, without raising the risk of overlooking concerning lesions [13, 17]. Based on the suggested algorithms, depending on the patients age, symmetrical flat lesions can be monitored until stabilization, symmetrical nodular lesions should be excised or scheduled for a close follow-up, while asymmetric Spitzoid tumors should always be excised [17].

Data from a newer study by Herzum et al. [15] also suggest that pediatric patients older than 12 years with clinically pigmented, flat, and lower-limb lesions had benign lesions in 97%, 98%, and 89%, respectively, and the simultaneous presence of all three criteria was always associated with benign histology (100%). This could thus probably represent a criterion for conservative management in pediatric patients older than 12 years [15].

Most recent studies also show that nodular spitzoid lesions at any age with dermoscopical multicomponent pattern should raise intense suspicion being highly associated with AST/SM and demand highest attentiveness [7, 15].

Newer studies also confirm that Spitzoid lesions in adult patients should always be closely monitored or excised as they tend to have a greater likelihood of malignancy [17].

After reviewing the most recent literature on this subject, as well as based on our own professional experience, we strongly advise outmost caution when dealing with a patient with spitzoid lesion, especially in those with nodular lesions and older than 12 years; in that light we developed our own protocol in managing spitzoid lesions (Figure 8).

With the following case series, we report clinical, as well as dermoscopic features of biologically various spitzoid lesions, appearing in different age groups. We believe that this case series may increase knowledge of physicians, pediatricians, and dermatologists about when to react when dealing with a patient with a Spitzoid lesion.

ACKNOWLEDGEMENT

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Conflict of interest: None declared.

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Клинички и дермоскопски спектар спицоидних лезија у односу на узраст пацијента – када реаговати?

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САЖЕТАК

Увод Спицоидне лезије представљају спектар меланоцитних лезија које обухватају бенигне спиц невусе, интермедијарне лезије познате као атипични спиц тумори и спицоидни меланом. Иако чешћи код деце и у млађој популацији, могу се јавити у било којој узрасној групи. Због сложености њихове клиничке, дермоскопске и хистопатолошке слике, изузетно су тешки за лечење, поготово у педијатријској популацији.

Приказ болесникâ У овом приказу представљамо серију од шест болесникâ са спицоидним лезијама у различитим старосним групама и са различитим исходима.

Закључак У раду приказујемо клиничке и дермоскопске карактеристике биолошки различитих спицоидних лезија, које се јављају у различитим старосним групама. Верујемо да ће овај чланак повећати знање како дерматолога, тако и других лекара о томе када и како реаговати када се ради о пацијенту са спицоидном лезијом на кожи.

Кључне речи: спицоидне лезије; спиц невус; атипични спиц тумор; спицоидни меланом; лечење

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REVIEW OF LITERATURE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Wilson's disease

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SUMMARY
Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism caused by mutations in the *ATP7B* gene, which is located on chromosome 13q14.3. The global genetic prevalence of WD at birth is approximately 13.9–15.4 per 100,000 population. Although WD is a rare condition associated with treatment efficacy, mortality rates in patients with WD (5–6.1%) are higher than healthy controls. Prevalent features of WD include hepatic, neurologic, and psychiatric syndromes, even though various signs and symptoms of the disease have been depicted to this point. If diagnosed and treated at an early stage, WD patients would likely improve and be often largely asymptomatic for the rest of their lives. Prompt diagnosis and lifelong treatment substantially affect outcome.
We aimed to summarize current knowledge about WD epidemiology, genetics, clinical manifestations, diagnostic workup, and current WD management.
Keywords: Wilson's disease; copper; diagnostic algorithms; treatment

INTRODUCTION

Wilson's disease (WD) is a rare, recessively inherited disorder of copper metabolism caused by its pathological accumulation in liver and extrahepatic organs [1]. Accordingly, prevalent features of WD include hepatic, neurologic, and psychiatric syndromes, even though various signs and symptoms of the disease have been depicted to this point. If diagnosed and treated at an early stage, WD patients would likely improve and be often largely asymptomatic for the rest of their lives. Prompt diagnosis and lifelong treatment substantially affect outcome.

We aimed to summarize current knowledge about WD epidemiology, genetics, clinical manifestations, diagnostic workup and current WD management.

WILSON'S DISEASE GENETICS

Wilson's disease is an autosomal recessive inherited disorder of copper metabolism caused by mutations in the *ATP7B* gene, which is located on chromosome 13q14.3. It encodes a copper-transporting P-type ATPase. Mutations in the *ATP7B* gene disrupt the synthesis and function of the *ATP7B* protein, thereby precipitating an impairment in the hepatocellular copper excretion pathway. Until now, 1275 mutations in the *ATP7B* gene have been identified [2]. The missense mutation H1069Q (substitution of histidine with glutamine on the position 1069) in exon 14 is the most common mutation responsible for WD worldwide. About 50–80% of WD patients from Central, Eastern, and Northern Europe carry at least one allele with the H1069Q mutation, with its highest frequency in Poland and Eastern Germany. The H1069Q is the most frequent mutation in Serbian population, found in 38.4–48.9% of analyzed alleles [3, 4].

The approach to identifying pathological mutations in WD focuses on targeting restricted exons known to contain the majority of mutations in one population [2, 3]. This strategy facilitates molecular genetic testing by eliminating the requirement for a protracted and expensive process.

EPIDEMIOLOGY

The global genetic prevalence of WD at birth is approximately 13.9–15.4 per 100,000 population [5]. Additionally, there is a difference in prevalence of WD between epidemiological (1.4 per 100,000) and genetic (13.9 per 100,000) studies. Finally, this disparity may be evidence of underdiagnosis of WD on a population level, or of delayed diagnosis and consequent early deaths [6]. Although WD is a rare condition associated with treatment efficacy, mortality rates in patients with WD (5–6.1%) are higher than healthy controls. Late diagnosis and stopping treatment might lead to a high mortality rate [7].

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CLINICAL PRESENTATION

In addition to the hepatic and neurological manifestations, whose significance is apostrophized through the name of 'hepatolenticular degeneration,' there is plenty of other symptoms and signs that result from the copper accumulation and cell damage in various tissues (Table 1). We will discuss common symptoms of WD in more detail.

Table 1. Clinical manifestations of Wilson's disease

Neurological manifestations	Extrapyramidal manifestations (tremor, parkinsonism, dystonia, chorea, myoclonus, tics)
	Cerebellar manifestations
	Dysarthria
	Dysphagia
	Other features (autonomic dysfunction, seizures, headaches)
Psychiatric manifestations	Personality changes
	Anxiety
	Depression
	Apathy
	Psychotic spectrum
	Mild cognitive impairment/Dementia
Hepatic manifestations	Chronic hepatic failure / cirrhosis
	Acute hepatic failure (including fulminant disease course)
Ocular manifestations	Kayser–Fleischer ring
	Sunflower cataract
	Other features
	nyctalopia, exorpic strabismus, optic neuritis, pallor of the papilla of the optic nerve, loss of accommodation
Other systemic manifestations	Hematologic (hemolytic anemia, thrombocytopenia)
	Myocardial (cardiac hypertrophy, arrhythmias, cardiomyopathy)
	Renal (hematuria, proteinuria, nephrocalcinosis, impairment of renal function)
	Musculoskeletal (osteoporosis, osteomalacia, osteoarthritis)
	Dermatological (hyperpigmentation, acanthocytosis nigricans, bluish discoloration of nails)

Neurological manifestations of Wilson's disease

While all untreated patients will develop neurological manifestations eventually, approximately 40–60% of patients present with neurological symptoms and signs at onset [8]. In patients with predominant neurological form of WD, first symptoms usually occur at around 20 years old, while patients have been described with first symptoms as early as six years old. Lately, special attention is drawn towards late-onset WD, suggesting that classic diagnostic approach should be modified and include testing for WD even for older individuals when high clinical suspicion is present.

Hallmark clinical manifestations of WD stem from basal ganglia involvement. Three major clinical syndromes were identified in patients with neurological symptoms at onset:

a) akinetic-rigid syndrome resembling parkinsonism,
b) generalized dystonic syndrome,
c) "pseudosclerotic" syndrome with predominant cerebellar manifestations.

Tremor is the most common neurological symptom affecting 30–50% of WD patients [9]. While coarse proximal tremor resembling wing-beating is typical for WD, these patients can present with static, kinetic, and intentional tremor. While arms are commonly affected, tremor can affect other body parts as well, mainly head and tongue. Besides tremor at rest, 40% of patients can present with other typical parkinsonian features, including rigidity and bradykinesia [10].

Dystonia (focal, segmental, multifocal, or generalized) is a presenting symptom in up to 69% of patients with neurological symptoms at onset [11]. MRI study showed that putamen was affected in 80% of WD patients with dystonia compared to only 24% in those without dystonia, suggesting its pathophysiological role in dystonia development [12]. Status dystonicus, life-threatening disorder characterized by acute worsening of generalized dystonia, can be induced in some WD patients either by introduction or withdrawal of specific drugs for WD [13, 14].

Cerebellar dysfunction is present in approximately 30% of patients and manifests as cerebellar tremor, appendicular ataxia, and speech and gait disturbances.

Speech disturbances are a pronounced feature of WD and in some case series, such as our own, the most common one, where 90% of patients had speech difficulties prior to treatment initiation [15].

Psychiatric manifestations of Wilson's disease

Most reports show that psychiatric symptoms occur as the earliest manifestation in about 30–40% of patients with WD [16]. The spectrum of these symptoms varies from subtle personality changes to frank psychosis. In our study of already established and treated WD, 72% of them had at least one psychiatric symptom on the neuropsychiatric inventory, while 44% had clinically significant symptoms [17]. Anxiety was the most common and the most severe symptom, followed by depression, apathy, and irritability [17].

Hepatic manifestations of Wilson's disease

Hepatic manifestation varies from subtle structural liver changes, which may be asymptomatic, to more severe presentations such as acute hepatitis, acute liver failure, recurrent jaundice and hemolysis, or cirrhosis [7]. Liver disease is a presenting symptom in about half (40–60%) of WD patients [7]. Usually in children and young adults there is mild to moderate liver disease with mildly elevated liver enzymes, witch if left untreated may progress to chronic liver disease with consequent cirrhosis, portal hypertension, hepatosplenomegaly, coagulopathy, and hypoalbuminemia. Hepatic decompensation may lead to ascites, jaundice, gastrointestinal bleeding due to coagulopathy and esophageal varices and hepatic encephalopathy with hyperammonemia [7]. The most severe form of

hepatic involvement in WD patients is acute liver failure often associated with hemolytic anemia, coagulopathy, encephalopathy, and rapid renal failure. Acute or rapid liver deterioration may occur in those WD patients who stopped treatment [7]. Most severe forms of liver disease may require liver transplantation [7].

Ocular manifestations of Wilson's disease

The most common ophthalmological manifestations of WD are Kayser–Fleischer's (KF) ring and sunflower cataract, but in this disease pathological changes can also be present in the retina, visual pathways, and in the ocular motility [18].

The KF ring is considered a pathognomonic sign of WD, consisting of concentric deposits of copper in Descemet's membrane at the very periphery of both corneas. It occurs in as many as 90.4–100% of people with a neurological form of WD, and somewhat less often (in 50–60% of cases) in those without neurological signs of this disease. This sign is considered not only essential for the diagnosis of WD, but also important for treatment and as a prognostic factor of the disease [7].

Sunflower cataract is a somewhat rarer manifestation of WD, as it occurs in about 2–20% of WD patients [7]. This lens opacification is yellowish in color, located under the lens capsule, with a central disc and radiating leaves that resemble a sunflower flower. It generally does not cause a significant decrease in visual acuity.

Other ophthalmological problems have been described in WD, but mostly sporadically – nyctalopia, exorpic strabismus, optic neuritis, pallor of the papilla of the optic nerve, loss of accommodation [7]. We found that WD patients may have lower intraocular pressure compared to the healthy population [19].

DIAGNOSIS OF WILSON'S DISEASE

Wilson's disease diagnosis is based on a combination of clinical features and laboratory parameters (Table 2) [7]. Multiple clinical, radiographic, and laboratory biomarkers of the WD when combined may facilitate the diagnosis, but the diagnosing process may be challenging because none of these biomarkers is specific to WD [1]. The first two steps should be liver disease laboratory assessment and ophthalmological examination for KF ring, followed by copper metabolism tests. If necessary, liver biopsy should be considered in selected cases. Genetic analysis is necessary for definite WD diagnosis, but still not widely accessible.

1. Laboratory testing should begin with clinical biochemical liver tests, blood counts, and coagulation parameters to assess for liver disease.

2. The KF ring is visible on slit lamp examination or anterior segment optical coherence tomography, rarely by naked eye. Absence of KF ring does not exclude WD diagnosis: it can be found in most cases with neurological form and in approximately half of cases with hepatic form of WD. Similar rings can be found in other chronic hepatic disorders, long lasting cholestasis, and cirrhosis [20, 21].

3. Copper metabolism

a. Serum ceruloplasmin

The normal concentration of ceruloplasmin measured by the enzymatic assay varies among laboratories (with a lower limit 0.15–0.2 g/L) [1]. In WD, ceruloplasmin is usually lower than 0.1 g/L [1]. However, this is typical value for neurologic WD, but it can be normal in about half patients with active liver disease [7, 22]. Modestly subnormal levels of ceruloplasmin (0.14–0.2 g/L) may be detected in heterozygotes [22]. Ceruloplasmin levels are increased in acute inflammation, estrogen supplementation and pregnancy, and decreased in malabsorption syndromes, renal or enteric protein loss, severe liver disease of any etiology and in a distinct genetic disorder – aceruloplasminemia [1].

b. Serum copper

Total serum copper consists of copper incorporated in ceruloplasmin and "free" serum copper which is not incorporated in ceruloplasmin. Total serum copper is usually decreased in proportion to reduced ceruloplasmin levels. Therefore, normal or increased total serum copper in individuals with decreased ceruloplasmin indicate an increase in the concentration of "free" serum copper. In most untreated

Table 2. Routine tests for diagnosis of Wilson's disease (adopted from references [1, 21])

Test	Typical finding	False-negative	False-positive
Serum ceruloplasmin	Decreased by 50% of lower normal limit	• Normal levels in patients with marked hepatic inflammation • Overestimation by immunologic assay • Pregnancy, estrogen therapy	Low levels in patients with malabsorption, malnutrition, aceruloplasminemia and in heterozygotes
24-hour urinary copper	> 100 µg (1.6 µmol) / 24 hours (> 40 µg (0.64 µmol) / 24 hours in children)	Normal Incorrect collection, children without liver disease	Increased: hepatocellular necrosis, cholestasis, contamination
Serum free copper	>100 µg/L (1.6 µmol/L)	Normal if ceruloplasmin overestimated by immunologic assay	
Hepatic copper	>250 µg (4 µmol)/g dry weight	Due to regional variation • In patients with active liver disease • In patients with regenerative nodules	Cholestatic syndromes
Kayser–Fleischer rings by slit-lamp examination	Present	Absent • In up to 50% of patients with hepatic Wilson's disease • In most asymptomatic siblings	Other chronic hepatic disorders with long lasting cholestasis and cirrhosis (i.e. primary biliary cirrhosis)

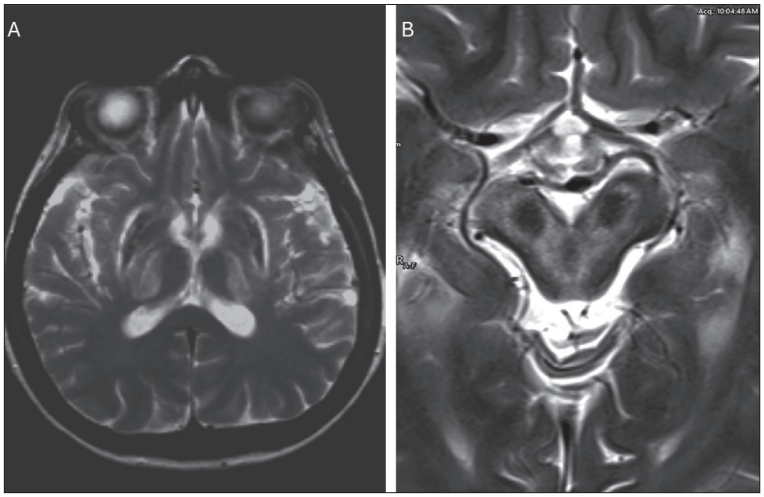

Figure 1. Symmetrical T2W hyperintense lesions of the lateral putamen; heterogeneous lesions of both thalami, hyperintense at the periphery and hypointense centrally; (A) "face of the giant panda sign" of mesencephalon when the substantia nigra and red nucleus are surrounded by a high T2W signal in the tegmentum (B); courtesy of Robert Semnic, MD

patients, "free" serum copper is elevated above 200 μg/L. Measuring serum copper is more important to monitor pharmacotherapy than in the diagnosis of WD [1, 7].

c. Urinary copper

In untreated symptomatic patients urinary copper levels > 100 μg / 24 h (> 1.6 μmol / 24 h) are considered diagnostic for WD. However, up to 23% of patients (mostly children and asymptomatic siblings) may have copper levels less than 100 μg / 24 h at the presentation. Keep in mind that urine copper may be elevated in other hepatic diseases and somewhat elevated in heterozygotes. Incomplete urine collection, copper contamination and renal failure may also affect measuring copper values. The radioactive copper test is available in highly specialized centers and is used if other cooper test or genetic findings are inconclusive [1, 7, 22].

1. Liver biopsy

A liver biopsy is sometimes needed for the differential diagnosis of liver pathology. In terms of the WD diagnosis, liver biopsy is used to measure hepatic parenchymal copper concentration. Hepatic copper > 250 μg (4 μmol)/g dry weight is considered diagnostic for WD [7].

2. Neuroimaging

Brain MRI is very important for WD diagnosis and may be helpful for treatment monitoring [7]. Typical finding is symmetric T2-weighted hyperintense changes in basal ganglia (mainly putamen and caudate nuclei), thalami, midbrain, and pons. In more advanced cases, these abnormalities can be seen in T1-weighted images as hypointense changes [7]. The 'face of the giant panda' in the midbrain is a characteristic sign, but present in up to 20% of cases of neurological WD (Figure 1) [1, 7]. Characteristic brain MRI changes occur in almost all drug-naive neurological WD patients, in 40–75% of hepatic and in 20–30% of presymptomatic WD patients [7], and are always symmetrical [23]. We studied 16 neurologically asymptomatic patients with hepatic form of WD and found that MRI showed brain parenchyma lesions in all untreated, and in 44% of treated patients [24]. In our study, prominent midbrain atrophy was present in patients with WD, with some measures changed regardless of the form of the disease (neurologic *vs.* hepatic). Some of the brain morphometric measures (interpeduncular angle, transverse diameter of the midbrain peduncle, and interpeduncular distance) were useful in differentiating patients from healthy subjects (probability reaching 93%) [25].

Transcranial sonography is a reliable and sensitive tool in detecting basal ganglia abnormalities, including accumulation of copper and other trace metals in WD [26]. We found significantly higher prevalence of hyperechogenicity in lenticular nucleus and substantia nigra in comparison with healthy controls [26].

3. Genetic analysis

The entire *ATP7B* gene sequencing should be performed if the diagnosis is difficult to establish by clinical and biochemical testing. Specific analysis for known mutations is recommended for WD screening in the first-degree relatives of WD proband [22].

WILSON'S DISEASE TREATMENT

The aim of WD treatment is to remove the accumulated copper from tissues and to prevent further copper gain by its increased elimination and/or reduced resorption of dietary copper (Table 3).

1. Dietary copper restriction

Copper is ubiquitous in food and water supplies and a low-copper diet has long been considered important in WD management. However, according to the current recommendations, a low-copper diet is unnecessary although some clinicians suggest avoidance of copper-rich foods such as shellfish, liver, cocoa, nuts, chocolate, mushroom, and dried fruits in first few months of treatment [27].

2. Chelation therapy

Chelating agents mobilize intracellular copper into the circulation and enhance its urinary excretion [28]. The most used chelating agents are penicillamine and trientine.

• Penicillamine

Penicillamine is a thiol with a sulfhydryl group that binds copper and facilities its excretion into urine. In

Table 3. Dosing, adverse effects and treatment monitoring for current Wilson's disease treatment (adapted according to references [27, 29])

Treatment	Symptomatic adults	Adults with severe neurological symptoms*	Maintenance dose (typically after 2 years)	Children	Adverse effects	Target for urinary copper excretion (typically after 9–12 months)
Penicillamine	start with 250 mg/day, increase dose weekly up to 1000–1500 mg/day in two divided doses	start with 125 mg/day, slowly increasing by 125 mg per 1–2 weeks, up to 1000–1500 mg/day in two divided doses	750 mg–1000 mg/day	125 mg/day slow increase by 125–250 mg per week to 20 mg/kg/day in two divided doses	early: • hypersensitivity reactions (fever and rash), proteinuria, bone marrow suppression (thrombocytopenia and neutropenia), • neurological worsening late: • lupus-like syndrome, Goodpastures syndrome, elastosis perforans serpiginosa, cutis laxa, poor wound healing	approximately 200–500 µg / 24h (3–8 µmol/24h)
Trientine	800–1600 mg/day for trientine dihydrochloride (Cufence); 450–975 mg/day for trientine tetrahydrochloride (Cuprior) in two divided doses	start with 150–200 mg/day, slowly titration by 150–200 mg per 1–2 weeks up to 800–1600 mg/day for trientine dihydrochloride (Cufence) or 450–975 mg per day for trientine tetrahydrochloride (Cuprior) in two divided doses.	800–1600 mg/day for trientine dihydrochloride (Cufence) or 450–975 mg/day for trientine tetrahydrochloride (Cuprior) in two doses	150–200 mg/day, slowly increasing by 150–200 mg/week to 400–1000 mg/day for trientine dihydrochloride (Cufence) or 225–600 mg/day for trientine tetrahydrochloride (Cuprior) in two divided doses	Early • urticaria or other rashes, arthralgia, myalgia, proteinuria, hematuria, sideroblastic anemia • neurological worsening	approximately 150–500 µg / 24 h (2.4–8 µmol / 24 h)
Zinc	150 mg/day of elemental zinc** divided in 2–3 doses	150 mg/day in 2–3 divided doses	150 mg/day in 2–3 divided doses	25 mg / day in patient < 6 years; 75 mg / day mg divided in three doses in patients aged 6–16 years or < 50 kg; 150/day mg divided in three doses in patient > 16 years or > 50 kg	early nausea, abdominal pain, gastritis, and paradoxical neurological worsening	< 100 µg / 24 h (< 1.6 µmol / 24 h)

*Generalized dystonia, oromandibular dystonia with dysphagia;
**Doses are for elemental zinc; therefore, true dose of zinc salt may differ and should be calculated

recommended doses of 1–2 g per day it initially leads to up to 9 mg of cupriuresis per day. Typically, by the end of 12–18 months after treatment introduction, penicillamine-induced copper excretion decreases following decrement of tissue accumulated copper [27]. According to the recommendations of recent therapeutic guidelines and our own experience, penicillamine is recommended for use in symptomatic patients during the initial intensive phase of treatment and later as maintenance therapy [15, 27, 29, 30]. It could also be recommended in presymptomatic patients. Women with WD should be made aware of the importance of continuing therapy during pregnancy, as stopping treatment may be associated with clinical deterioration [27]. However, breastfeeding should be avoided because penicillamine is excreted in breast milk and may interfere with the infant's copper metabolism [27]. In symptomatic adults, recommended penicillamine dose is 1–1.5 g per day (the dose in children is 20 mg/kg/day) [27, 29]. Given that most adverse effects are dose-dependent, the general rule is to start low and go slow, aiming to reach the initial target dose in 6–8 weeks. We usually start with 125–250 mg per day with dose increments of 125 mg per week in adults presenting with neurological symptoms.

Although it is not necessary, we usually admit patient to the hospital to initiate treatment because careful monitoring for adverse effects is essential, including neurological follow-up, full blood count, liver function tests and renal profile every week during the first month of treatment initiation. Then, in outpatient settings, we advise monitoring the mentioned parameters once a month during the first six months of therapy. After establishing sustainable clinical improvement, which is estimated to require about two years of continuous use of chelators, the dose may be reduced to 500–750 mg per day to prevent disease progression. According to The Wilson's Disease Support Group UK, 24-hour urinary copper output while continuing medications (on treatment) should be 3–8 µmol / 24 h (200–500 µg / 24 h) with chelating agents [27].

On the basis that high doses of penicillamine may disrupt pyridoxine metabolism, prophylactic supplementation

with 50 mg once a day is usually advised [2, 7, 30]. D-penicillamine should be taken twice a day on an empty stomach, preferably fasting 3–4 hours before and 2–4 hours after the dose.

Up to 30% of WD patients with neurological symptoms at presentation may develop paradoxical neurological worsening after penicillamine treatment introduction. It may occur within the first six months after treatment initiation [31]. Sometimes deterioration is irreversible or even fatal [14].

• Trientine

The four amino groups of trientine form a stable ring complex with copper and facilitate cupriuresis. The efficacy of trientine and penicillamine regarding urinary copper excretion is similar. Trientine is a life-saving treatment option in patients in whom penicillamine had to be discontinued due to adverse events or in patients who have increased risk of adverse effects (i.e. history of autoimmune diseases, severe thrombocytopenia, or renal disease and allergy to penicillin) [1, 27]. Trientine could be used as a first-line therapy in the initial intensive and the later maintenance phase of treatment in both symptomatic and asymptomatic WD patients; it is safe to use during pregnancy but should be avoided during breastfeeding [27].

• Zinc salts

Zinc salts reduce intestinal absorption of copper. Zinc salts given orally are absorbed by the intestinal cells, in which they increase production of metallothionein, cysteine-rich protein that can bind various metal ions [29]. Subsequently, metallothionein-bound copper is eliminated by feces when the enterocytes are shed in the intestinal lumen during physiological intestinal cell turnover [27, 29]. According to a recent guidance, zinc is recommended as maintenance therapy in symptomatic patients once symptoms have regressed following treatment with oral copper chelators and as the first-line therapy in asymptomatic patients [27]. Zinc is administered as sulfate, acetate, or gluconate salts. The recommended dose is 150 mg of elemental zinc in adults, or 75 mg in children, in two or three divided doses per day. Food interferes with zinc absorption – therefore, it should not be given at mealtimes. Zinc can be safely used during pregnancy [27, 29].

LONG TERM OUTCOME

Patients with WD who are timely diagnosed and compliant to de-coppering treatment have favorable disease outcome. The treatment adherence is a single most important determinant of a good long-term disease prognosis [7, 15]. However, despite overall good response to de-coppering treatment, a significant proportion of patients feature residual neurological symptoms [32, 33]. In our patients with neurological form of WD who had good therapy adherence, presence of dystonia at the disease onset was a predictor of residual disability, in line with other studies suggesting that dystonia is a poor indicator of both short- and long-term outcome [15, 33]. The extent to which the diagnostic and treatment delay negatively affects prognosis varies among studies [15, 34]. A four-fold increased mortality rate compared to the general population was reported with cumulative mortality over 10 years of 9.3% in WD compared to the mortality rate of 2.4% in reference healthy individuals [35]. Principal causes of death in WD include long diagnostic delay, non-compliance to the therapy, and the development of malignancies [36]. In our cohort of 142 patients, the most frequent causes of death were liver failure due to cirrhosis in 16.6% and hemorrhage due to esophageal varices in 13.3% of patients, while a surprisingly high rate of suicide (13.3%) was observed, with mortality rate due to suicide being 1.7 times higher compared to the age-matched Serbian population [37].

Both mental and general health were among the Quality of Life (QoL) dimensions mostly affected in WD [38]. QoL is poor in patients who experienced a long delay in treatment and generally worse in patients with neurological form of the disease than in patients with hepatic form [39]. Psychiatric symptoms (in particular depression) were identified as a significant determinant of poor QoL in WD [39, 40]. A lack of standardized QoL assessment scale and more studies with larger sample sizes represent an unmet need in addressing QoL in WD [37, 38].

CONCLUSION

WD is a treatable neurovisceral disorder not to be missed. There are numerous clinical, biochemical, and imaging biomarkers which, combined, facilitate a timely diagnosis of WD. Prompt therapy aimed to reduce copper overload should be immediate, if possible, even in asymptomatic individuals. Early treatment is connected to favorable outcome, it could prevent further progression of symptoms, may reduce present symptoms and significantly influence patients' quality of life.

Ethics: The authors declare that the article was written according to ethical standards of the Serbian Archives of Medicine as well as ethical standards of institutions for each author involved.

Conflict of interest: Marina Svetel has received speaking honoraria from Makpharm, Salveo, Unihem and Pharmaswiss. Nikola Kresojević received speaking honoraria from Makpharm, Salveo, Remedica, and travel grants from Vemax Pharma, Salveo, Goodwill Pharma. Aleksandra Tomić has received speaking honoraria from Makpharm, Unihem, and travel grant from Medtronic. Milica Ječmenica Lukić and Vladana Marković received speaking honoraria from Makpharm. Nataša Dragašević and Igor Petrović received speaking honoraria from Makpharm and Salveo. Iva Stanković, Tatjana Pekmezović, Ivana Novaković, Marija Božić, Marko Svetel, and Jelena Vitković have nothing to report.

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Вилсонова болест

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САЖЕТАК

Вилсонова болест (ВБ) је аутозомно рецесивни наследни поремећај метаболизма бакра узрокован мутацијама у гену *ATP7B*, који се налази на хромозому *13q14.3*. Глобална генетска преваленција ВБ при рођењу је приближно 13,9–15,4 на 100.000 становника. Иако је у питању ретка болест за коју постоји ефикасна терапија, стопе морталитета код болесника са ВБ (5–6,1%) веће су од здравих контрола. Доминантне карактеристике ВБ укључују хепатичне, неуролошке и психијатријске синдроме, а поред њих су описани бројни други симптоми и знаци. Ако би се дијагноза поставила и лечење започело у раној фази, стање болесника са ВБ би се вероватно побољшало и болесници би често били углавном без симптома до краја живота. Правовремена дијагноза и доживотно лечење значајно утичу на исход.

Циљ нам је био да сумирамо тренутна знања о епидемиологији ВБ, генетици, клиничким манифестацијама, дијагностичкој обради и тренутном управљању ВБ.

Кључне речи: Вилсонова болест; бакар; дијагностички алгоритам; лечење

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REVIEW OF LITERATURE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Association of common glutathione transferase polymorphisms with ovarian cancer risk and chemoresistance

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SUMMARY
Ovarian cancer is regarded as the most lethal gynecological cancer with a five-year survival below 45%. It represents the seventh most common cancer among women. Due to the limited availability of biomarkers and reliable screening methods for early diagnosis of ovarian cancer, much research is being conducted to explore and understand the factors that may increase the risk of developing this kind of cancer. When surgery and chemotherapy treatments have been fully utilized, the development of chemoresistance becomes a critical factor in the progression of the disease. Glutathione transferases (GSTs) are a group of enzymes that play a role in the process of detoxification. Genes that code for GSTs proteins exhibit polymorphism, which can lead to either total or partial loss of enzymatic function. Cytosolic GST activity is composed of many different isoenzymes that facilitate interactions between glutathione and hazardous chemicals, including cancerogenes, anticancer drugs, and byproducts of oxidative stress. The scope of this review is to clarify the association of common GST polymorphisms with ovarian cancer risk and chemoresistance.
Keywords: glutathione transferases; ovarian cancer; polymorphisms; risk; chemoresistance

OVERVIEW OF OVARIAN CANCER

Ovarian cancer is regarded as the most lethal gynecological cancer with a five-year survival below 45%. Around 290,000 women worldwide are diagnosed with ovarian cancer and 180,000 die every year, which represents the seventh most common cancer among women [1, 2, 3]. Ovarian cancer can originate from any of the histologic components of the ovary, such as epithelium, stroma, or germ cells. The most prevalent among them is high-grade serous ovarian carcinoma (HGSOC) originating from the ovarian epithelium [4]. However, pathogenesis of ovarian cancer has evolved from the hypothesis that HGSOC develops from ovarian epithelium to the theory that it starts as a precursor lesion in the epithelium of distal fallopian tube as a serous tubal intra epithelial carcinoma [5, 6]. Almost 15–20% of HGSOC have germline *BRCA* ½ mutations with cumulative risk of epithelial ovarian cancer estimated to be 44% and 17% for *BRCA1* and in *BRCA2* mutation carriers, respectively [7].

Over the course of time, several distinct risk factors have been discovered for ovarian cancer. As an example, La Vecchia [8] examined the connection between the age at which menstruation begins, the age at which menopause occurs, the regularity of ovulation cycles, and the specific locations of ovarian cancer. Researchers determined that women with irregular menstrual cycles had a nearly 42% greater likelihood of developing ovarian cancer during the postmenopausal phase of life [8]. This may also be observed from the perspective of polycystic ovarian syndrome, which is considered the most common cause of irregular menstrual periods in women of reproductive age [9].

From the pathology standpoint of view, HGSOC has a diverse development pattern characterized by the presence of huge papillae, glandular structures, solid areas, and occasional micropapillary formations, sometimes accompanied by necrosis [10]. It is distinguished by the presence of high-grade nuclei, and a high mitotic index. The immunohistochemistry stain usually shows aberrant expression of p53, diffuse expression of p16, and high expression of Ki67. Other markers associated with HGSOC are ER, PR, WT-1, and PAX8 [11].

Since, HGSOC is regarded as chromosomally unstable with frequent DNA gains and losses, there is a great deal of potential for acquiring chemoresistance [12]. Molecular abnormalities are defined by ubiquitous inactivating mutations in TP53, copy number alterations and whole genome duplications [11]. From a molecular perspective, high grade ovarian cancer can be classified into four distinct subtypes: mesenchymal, immunological, differentiated, and proliferative [13, 14]. Research done by Cheng et al. [15] discovered seven copy-number signatures that are associated with the prognosis of ovarian cancer.

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EPIDEMIOLOGY AND INCIDENCE

The average lifetime risk of developing ovarian cancer is 1.3%, the equivalent of 1 in 78 women [16]. The overall ovarian cancer incidence rate in the US was 11.5 per 100,000 women during 2010–2014. More than 90% of ovarian malignancies are of epithelial origin. Epithelial ovarian carcinomas are classified by tumor cell histology as serous, endometrioid, mucinous, or clear cell, with one-quarter being more rare subtypes or unspecified.

At the moment, there is no officially endorsed screening test for ovarian cancer. However, there are extensive randomized clinical trials aimed at identifying viable screening methods. In fact, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial evaluated the effectiveness of transvaginal ultrasound and detection threshold (≥ 35 U/mL) in the tumor marker CA125 for detecting ovarian cancer at an early stage. But the study did not find any decrease in ovarian cancer-related deaths even after a follow-up period of up to 19 years [17].

CHALLENGES IN DIAGNOSIS AND TREATMENT

Nonspecific symptoms such as abdominal bloating, early satiety, nausea, and abdominal distension are associated with ovarian cancer. Changes in bowel function, urinary symptoms, fatigue, and weightloss usually manifest many months before the diagnosis is made. The majority of patients are diagnosed in an advanced stage of illness. Due to the extended period of asymptomatic nature of the disease, signs of ovarian cancer are demonstrated by the pattern of cancer tissue spreading throughout the abdominal cavity.

The classic treatment method of ovarian cancer is characterized by an initial debulking surgery followed by chemotherapy [18]. The most crucial factor for survival is the complete elimination of any visible illness in the abdomen [19]. Poor prognosis and inferior survival rates are associated with suboptimal surgical treatment that fails to completely eradicate the tumor burden.

ROLE OF GLUTATHIONE TRANSFERASES IN CANCER

Cytosolic GST is made up of several different forms of enzymes that facilitate interactions between glutathione and lipophilic molecules containing electrophilic centers. GSTs are recognized as important enzymes in cell detoxification processes, playing a critical role in the metabolism of both external substances (such as chemical carcinogens, environmental contaminants, and even anticancer drugs) and internal electrophilic chemicals [20]. Regarding the substrates of GSTs, it is important to mention that GSTM1, GSTP1 and GSTT1 are involved in the inactivation of substances that can cause cancer, produced during the breakdown of polycyclic aromatic hydrocarbons and arylamines [21]. GST family members also possess antioxidant activity [22, 23].

GST enzymes have been categorized into seven classes: alpha, mu, pi, theta, zeta, omega, and sigma. The most extensively investigated glutathione transferase genetic polymorphisms are GSTA1, *GSTM1*, GSTP1, and GSTT1 and to some less extent GSTO1 [24]. Most of the variations found in genes that code for cytosolic GSTs are single nucleotide polymorphisms (SNPs). The substitution of isoleucine (Ile) with valine (Val) resulting from SNP alters the catalytic and regulatory characteristics of the GSTP1 enzyme [25, 26]. On the other hand, the GSTA1 polymorphism is characterized by three SNPs, namely -567TOG, -69COT, and -52GOA, which appear to be connected. The changes mentioned lead to differential expression, with the variant GSTA1*B allele (-567G, -69T, -52A) having reduced transcriptional activation compared to the common GSTA1*A allele (-567T, -69C, -52G) [27]. The substitution of the amino acid Alanine (Ala) with Aspartic acid (Asp) at position 140, caused by SNP where C is replaced by A, in exon 4 of the GSTO1 gene (GSTO1*Ala140Asp), alters the deglutathionylase and thioltransferase activity of the gene [28, 29, 30]. The GSTO2 rs156697 polymorphism involves a SNP where the nucleotide A is replaced by G. This substitution results in the amino acid Asparagine being replaced by Aspartic acid at position 142 (GSTO2*Asn142Asp) [31, 32]. This genetic variation may be associated with changes in the levels of the GSTO2 protein.

The functional importance of GST SNPs has been emphasized by Hollman et al. [33], who proposed a categorization of disorders strongly associated with SNPs discovered in GSTs, including malignancies. Conversely, deletion polymorphisms of genes that encode for human cytosolic GSTM1 and GSTT1 are rather prevalent in human populations. Roughly 50% of the population does not have GSTM1 enzyme activity because they have a homozygous deletion of the *GSTM1* gene. In the case of GSTT1, about 20% of Caucasians have a gene homozygous deletion, resulting in a lack of GSTT1 enzyme activity [34, 35].

GST polymorphisms have been associated with an increased risk for a variety of other types of cancer, such as breast, gastric, renal, lung and colorectal [36]. Meta-analysis conducted by Chinese authors on total of 17 studies, involving 5323 breast cancer cases and 7196 controls, found a significant association between the null *GSTM1* and breast cancer risk [37]. This study exclusively recruited individuals of Chinese ethnicity, which limits the ability to apply the findings on general population due to the well-established variations in the distribution of *GSTM1* among other racial populations. The frequency of GSTT1 null genotype varies from 20% in Caucasians to 60% in Asians, whereas around 50% of the global population (varying from 22% in Africa to 62% in Europe) have the *GSTM1* null genotype [21].

A study carried out by Coric et al. [38] examined the impact of GST gene variations on the likelihood of developing renal cell carcinoma (RCC) and the postoperative prognosis in patients with clear cell RCC. It has been shown that individuals with *GSTM1*-null and GSTP1-variant genotypes have a higher risk of developing RCC. In contrast, the absence of the GSTM1 protein due to *GSTM1* null genotype is linked to a positive postoperative outcome in clear cell RCC [38]. Matic et al. [39] investigated

the correlation between genetic variations in the GSTA1, *GSTM1*, GSTP1, and GSTT1 genes with the risk of bladder cancer. They further assessed whether this correlation was influenced by smoking. The presence of null or low-activity genotypes of the GSTA1, *GSTM1*, GSTT1, and GSTP1 genes did not have a separate impact on the likelihood of developing bladder cancer in our patients. Nevertheless, the combination of low activity GSTA1 and *GSTM1*-null genotype enhances an individual's vulnerability to bladder cancer when associated with smoking [39].

In addition to its typical function as a phase II detoxifying enzyme, glutathione transferases may also directly inactivate several standard anti-cancer medications (such as chlorambucil, cyclophosphamide, melphalan, cisplatin, thiotepa, and others] through GST-dependent conjugation reactions, as they are also substrates for GSTs [40, 41]. GSTs also appears to interact with efflux transporters, hence enhancing the efflux of anticancer drugs from the cell. Additionally, GSTs interact with other signaling molecules that are involved in the control of apoptosis. This specifically applies to the GSTP1, since it can bind to both small and large molecules. It acts as a suppressor of kinase-dependent apoptotic signaling pathways by forming protein-protein complexes with regulatory mitogen-activated kinases like JNK1 (c-Jun NH2-terminal kinase). Additionally, GSTP1 plays a role in detoxifying potentially cancer-causing substances. Furthermore, it can enhance the removal of drugs from cells, thereby contributing to chemoresistance. Also, GSTP1 demonstrates a synergistic impact on the development of chemoresistance to ethacrynic acid, chlorambucil, vincristine, and etoposide via interacting with MRP-1 [42].Thus, the catalytic and regulatory activities of GSTs may be regarded as significant components that contribute to at least several key pathways of chemoresistance [20]. Obviously, GSTs may have a role in chemoresistance, even for drugs that are not typically metabolized by GSTs. Therefore, GSTs appear to be well-suited for the creation of new drugs, particularly because each type of cancer cells possess a distinct GST signature. This allows for precise targeting and selectivity when designing inhibitors and pro-drugs specific to each isoenzyme [43, 44]. Ishikawa et al. [45] carried out research on the expression of ATP-dependent glutathione S-conjugate export pump (GSX-Pump) and the capacity of tumor cells to eliminate a potentially cytotoxic glutathione-platinum complex, thereby modulating glutathione (GSH)-associated resistance to cisplatin. It was discovered that human promyelocytic leukemia HL-60 (HL-60/R-CP) cells had functionally overexpressed GSX-Pump [45]. Horton et al. [46] performed a study on ovarian cancer cells that were isolated after subjecting the parent cells to increasing doses of alkylating agent over time. The study demonstrated that the resistant cell line exhibited nearly a five-fold increase in GST activity compared to the original cell line. Additionally, they demonstrated that the resistant cell line exhibited a minimum of 11 times greater GSTM activity compared to the parental cells, in which this isoform was hardly detectable. The results have shown a close and direct relationship between resistance to the alkylating drug chlorambucil and the increased expression of mu-class GSTs. These findings imply that this increased expression may be partially responsible for the acquired resistance of ovarian cancer cells [46].

GLUTATHIONE TRANSFERASE POLYMORPHISMS AND OVARIAN CANCER RISK

Due to the difficulty in the early-stage diagnosis of ovarian cancer, extensive research has been dedicated to understanding the factors that increase an individual's vulnerability to this malignant illness. Currently, there are no recommendations regarding population-wide screening procedures that have demonstrated sufficient effectiveness to be implemented in routine practice. SNPs are the predominant type of genetic variation in humans and might potentially influence an individual's susceptibility to cancer. It seems reasonable to assume that glutathione transferases play a role in metabolizing different carcinogens that could potentially contribute to the development of ovarian cancer, providing a strong biological basis for the study of associations of GST polymorphisms with risk of ovarian cancer. Several large-scale meta-analyses have been undertaken in recent decades to investigate the correlation between GST polymorphisms with the likelihood of developing ovarian cancer. Significantly, most of them focused on specific GST polymorphisms, including the GSTM, GSTT, and GSTP gene families. Economopoulos et al. [21] conducted a large and thorough meta-analysis. They analyzed eight studies that investigated the *GSTM1* null polymorphism status, with a total of 2357 cases and 3044 controls. They also analyzed six studies on the GSTT1 null polymorphism, with 1923 cases and 2759 controls. Additionally, three studies on the GSTP1 Ile105Val were included in the meta-analysis. The investigated *GSTM1*, GSTT1, and GSTP1 genetic polymorphisms do not appear to provide any extra proof of susceptibility to ovarian cancer [21]. In a study carried out by Jin et al. [47], they utilized literature data to examine the association between *GSTM1* polymorphism and GSTT1 polymorphism in ovarian cancer. They identified a total of eight studies, which included 2397 cases and 2910 controls for *GSTM1* polymorphism, and 2049 cases and 2668 controls for *GSTT1* polymorphism. The comprehensive data indicated that individuals with the *GSTM1* null genotype did not exhibit a substantially higher risk of ovarian cancer compared to those with the *GSTM1* active genotype. In both the overall analysis and the subgroup of Caucasian subjects, no association was found between *GSTT1* polymorphism and the investigated model [47]. Although current studies have not yet provided conclusive evidence of a link between GST polymorphisms and epithelial ovarian cancer risk, recent study on the role of GST omega class polymorphisms has shown that that *GSTO* locus variants may confer ovarian cancer risk. Preferably, *GSTO2* should be primarily sequenced for variants that may influence the disease risk [48, 49].

Nevertheless, investigations done on other cancers of the urogenital system indicate a correlation between

GSTs and the development of cancer. Matic et al. [39] investigated the correlation between genetic variations in the *GSTA1, GSTM1, GSTP1*, and *GSTT1* genes with the incidence of bladder cancer. Additionally, they assessed whether these variations were influenced by smoking. None of the analyzed polymorphisms exhibited a statistically significant independent connection with bladder cancer risk. However, when combined with smoking, both the low activity *GSTA1* and *GSTM1*-null genotype contribute to an increased vulnerability to bladder cancer [39]. These findings indicate that GSTs polymorphisms have a role in carcinogenesis, and lifestyle factors can also impact the outcome. Since this aspect was not included in the previous research on the risk of ovarian cancer, the study of gene-environmental interactions in ovarian cancer risk might be an appropriate direction for future investigations. Besides, polymorphisms of GST members should also be examined in correlation with the degree of oxidative stress which may be one of contributing factors that initiate ovarian cancerogenesis [22, 23, 50].

GLUTATHIONE TRANSFERASE POLYMORPHISMS AND OVARIAN CANCER CHEMORESISTANCE

The conventional treatment for ovarian cancer patients involves cytoreductive surgery followed by the administration of chemotherapeutic drugs, specifically platinum-based compounds with taxans. However, a significant number of patients who experience a relapse develop resistance to platinum-based chemotherapy due to repeated treatment cycles. As a result, resistance to chemotherapy, whether inherent or acquired, is a prevalent issue in the management of ovarian cancer patients. The precise mechanisms of chemoresistance have not yet been completely understood. Presently, about 90% of patients acquire a kind of chemoresistance that ultimately proves fatal. The primary factors contributing to chemoresistance are:

1. the fact that there are heterogeneous tumor cells;
2. the presence of cancer stem cells;
3. the particular features of the tumor microenvironment [51].

As previously mentioned, the majority of polymorphisms found in genes that encode cytosolic GSTs belong to SNPs. Furthermore, the alterations in amino acids caused by SNPs lead to variations in the expression of GST variants, resulting in reduced transcriptional activity or functional change due to altered protein structure [43]. In addition to their role in promoting chemoresistance through their conjugating activity, GSTs also appear to interact with efflux transporters, therefore enhancing the efflux of anti-cancer drugs from the cell. This is another mechanism that is related to the development of chemoresistance [42].

Zhang et al. [52] examined the molecular and cellular factors behind chemoresistance in ovarian cancer, focusing on determining the expression of genes that encode glutathione transferase T1 in ovarian cancer cell cultures. The study demonstrated a significant increase in the expression of GSTT1 genes in serous ovarian cancer cell lines that are resistant to paclitaxel and carboplatin [52]. In addition, the study done by Liblab et al. [53] examined the correlation between genetic variations in ERCC1, XRCC1, and GSTP1, which have a role in platinum metabolism. The study showed that individuals with the GSTP1 A/G genotype exhibited a greater incidence of grade 2 anemia. This finding suggests that grade 2 anemia might potentially serve as a valuable indicator for predicting the clinical effectiveness of platinum-based chemotherapy [53]. These conclusions display the intricate function that glutathione S transferase can play in oncological therapy. Both polymorphisms and gene expression can impact the outcome. In addition, it has been revealed that GST can also impact the rate of complications, which are classified based on the grade or severity of multiple organ systems as a secondary effect of chemotherapy.

Kolwijck et al. [54] assessed the correlation between GSTP1-1 levels in ovarian cyst fluid, collected prior to chemotherapy during surgery, and the clinical outcomes of patients with epithelial ovarian cancer. The study included a total of 56 patients diagnosed with epithelial ovarian cancer and 109 patients without disease, who served as controls. The outcome was assessed by comparing the duration of progression-free survival and overall survival. It was discovered that patients with malignant illness and advanced FIGO stage exhibited elevated levels of GSTP1. Also, patients who received chemotherapy and had elevated levels of GSTP1 have worse progression-free survival and overall survival rates. Therefore, it may be concluded that cancer cells increase their ability to metabolize and remove anti-cancer drugs in GSTP1 dependent manner [54]. Nagle et al. [55] examined the impact of glutathione-S-transferase polymorphisms on the survival of women diagnosed with ovarian cancer. The researchers examined Australian women who were diagnosed with ovarian cancer between 1985 and 1997. They used DNA isolated from peripheral blood and uninvolved (normal) tissues for their analysis. They found that women with non-functional GST polymorphism, specifically the GSTP1 Ile105Val GG/GA genotype, experienced a significantly improved survival. This can be attributed to the enhanced ability of functional GST enzymes to efficiently detoxify anti-cancer drugs, leading to quicker elimination and reduced impact on tumor cells [55]. The study undertaken by O'Brien et al. [56] examined the impact of coordinated overexpression of glutathione phase II detoxification gene products on drug resistance. Specifically, glutathione, glutathione transferases, and the multidrug resistance-associated protein 1 have been individually examined for their roles in drug resistance. Upon combining all three, there was a notable increase in resistance levels for doxorubicin and etoposide. These findings validate the idea that the simultaneous improvement of detoxification pathways leads to a more effective defensive characteristic, leading to enhanced survival of tumor cells [56].

Khrunin et al. [57] analyzed 21 variations in 10 genes that encode the proteins responsible for cisplatin metabolism. A study was conducted to examine the relationship between the effectiveness and harmful effects of the

cisplatin-cyclophosphamide treatment in 104 patients with ovarian cancer. The association between the *GSTP1* Ile105Val polymorphism and progression-free survival was shown to be significant. The allelic status of the *GSTA1* C > T polymorphism was shown to be associated with better overall survival. Despite this, there were no observed associations between genotypes and complete tumor responses [57]. Furthermore, Kim et al. [58] conducted an analysis on several genes, including *GSTP1, GSTM1,* and *GSTT1* polymorphisms, to investigate their relationship with drug toxicity and their potential as a predictive factor. Data was collected for the medical records of 118 patients. The study demonstrated that having an active (non-null) genotype in the *GSTT1* was associated with a lower likelihood of overall response to chemotherapy. Additionally, those with an *A/A* genotype in the *GSTP1* Ile105Val polymorphism had a significantly higher chance of experiencing grade 3 or 4 hematological complications. Both of these two studies highlight the significance of *GST* polymorphisms in the prognosis, responsiveness, and complication rate associated with ovarian cancer [58].

Nonetheless, several compounds that can inhibit GSTs have been produced, and certain natural inhibitors have also been identified and studied. This is because blocking GSTs can help reverse drug resistance. The synthesis of GST inhibitors and the investigation of natural inhibitors have been extensively documented. Most of these compounds are either GST substrates or GSH analogs or mechanism-based inhibitors, resulting in enzyme inhibition through various mechanisms. The efficient accumulation and/or activation of anti-cancer drugs within cancer cells can be achieved by exploiting the overexpression of certain GSTs in distinct types of malignancies. Therefore, GSTs are appropriate as biomarkers for combination therapy including specific GST inhibitors and for the creation of new anti-cancer medications with focused selectivity [43, 59]. Molecules with the ability to inhibit GSTs may play a role in the series of actions that can be taken to combat drug resistance. Specifically, a substantial quantity of GST inhibitors has previously been developed, while there has been long-standing evidence of natural inhibitors. The primary mechanisms underlying the reversal of drug resistance involve the creation or utilization of molecules that act as either GST substrates or GSH analogues, therefore leading to enzyme inhibition in many ways.

Collectively, the previously cited research papers suggest a strong association between GSTs polymorphisms and clinical response, specifically in terms of overall survival and prognosis of chemotherapy toxicities. Insufficient activity in GST enzymes seems to result in an improved response to therapy [60].

CONCLUSION – CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Glutathione transferases are essential for the detoxification of several conventional chemotherapeutic agents. Additionally, they are involved in the regulation of cellular proliferation and apoptosis. Conventional cancer treatment faces a significant obstacle in the form of inadequate capability to target cancer cells specifically while minimizing adverse effects and resistance to anticancer medications. Addressing the issue of multidrug resistance to chemotherapeutic agents is a significant concern in the field of ovarian cancer treatment as well. Similarly, considerable research effort is being devoted to discovering novel and inventive approaches to overcome this challenge. Likewise, chemoresistance is an exceedingly complex and multifaceted phenomenon that encompasses a multitude of underlying mechanisms. Introduction of new technology in conjunction with innovative GST targeted drugs offers innovative approaches to combating the growing trend of chemoresistance in ovarian cancer.

Ethics: The authors declare that the article was written according to ethical standards of the Serbian Archives of Medicine as well as ethical standards of institutions for each author involved

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Повезаност полиморфизама за глутатион-трансферазу са ризиком за оваријални карцином и појаву хемиорезистенције

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САЖЕТАК

Карцином јајника се сматра најсмртоноснијим гинеколошким карциномом са петогодишњим преживљавањем испод 45%. Он представља седми најчешћи рак међу женама. С обзиром на то да је доступност биомаркера карцинома јајника ограничена, као и да не постоје поуздане методе скрининга, у току су многа истраживања фактора који повећавају ризик за настанак овог тумора. Након исцрпљивања хируршких и хемиотерапијских метода лечења карцинома јајника, развој хемиорезистенције постаје критичан фактор у напредовању болести. Глутатион-трансферазе су фамилија ензима који играју кључну улогу у процесу детоксикације. У генима који кодирају глутатион-трансферазе постоје полиморфизми, који могу довести до потпуног или делимичног губитка функције ензима. Цитосолну активност глутатион-трансфераза чини много различитих изоензима који каталишу интеракције између глутатиона и токсичних једињења, укључујући канцерогене, лекове против рака и продукте оксидативног стреса. Циљ овог прегледног чланка је да разјасни повезаност најчешћих полиморфизама гена за глутатион-трансферазе са ризиком за настанак рака јајника и његовом хемиорезистенцијом.

Кључне речи: глутатион-трансфераза; карцином јајника; полиморфизми; ризик; хемиорезистенција

CONGRESS AND SCIENTIFIC MEETING REPORT
/ ИЗВЕШТАЈ СА КОНГРЕСА И НАУЧНОГ СКУПА

"Artificial Intelligence and Medicine" – joint symposium of the Academy of Engineering Sciences of Serbia and the Academy of Medical Sciences of the Serbian Medical Society

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The Academy of Engineering Sciences of Serbia (AESS) and the Academy of Medical Sciences of the Serbian Medical Society (AMS-SMS) have successfully organized a joint symposium titled "Artificial Intelligence and Medicine" held in Belgrade on May 15, 2024.

At the opening of the symposium, Professor Dr. Miloš Nedeljković, president of AESS and Professor Dr. Svetolik Avramov, president of AMS-SMS greeted the gathering. Both presidents expressed their satisfaction that the cooperation between the two academies was established in 2020 by signing a cooperation protocol. Professor Nedeljković reminded the audience that the first joint meeting between the two academies was held in 2021 with a series of lectures from various fields in which engineers and doctors collaborate [1, 2]. He added that the proposal for the symposium on artificial intelligence (AI) and medicine was made by Professor Dr. Ljubica Đukanović, former president of the AMS-SMS, who with Professor Dr. Aleksandra Smiljanić, vice president of AESS, prepared the program of the symposium. He congratulated the professors on the rich program and interesting lectures that would be held by doctors and engineers. He also expressed his satisfaction that the audience included engineers, physicians, dentists, biochemists, and pharmacists and that the symposium was accredited by the Health Council of Serbia. Professor Avramov expressed his satisfaction that the cooperation between the two academies, which was slightly hindered by the COVID-19 pandemic, continues. He emphasized the importance of cooperation between engineers and doctors, which contributed to the incredible development of medicine in recent decades [3, 4]. He especially praised the actuality of the topic of the symposium and added that AI is already being applied in several branches of medicine. There is no doubt that its application will lead to further and significant progress in medicine. Both presidents wished everyone a successful symposium and future joint scientific meetings between the two academies.

The program of the meeting consisted of 10 lectures covering different areas of medicine in which AI finds application.

Professor Dr. Vladan Devedžić (Faculty of Organizational Sciences, University of Belgrade), a corresponding member of the Serbian Academy of Sciences and Arts, gave the opening talk titled "If the band you are in starts playing different tunes: a tale about generative artificial intelligence." Professor started his talk with the explanation of what generative AI is, and how large language models, large visual models, and other similar AI models allow for generating different kinds of content. There are numerous specific applications that use such models, like the famous ChatGPT chatbot, the Midjourney image generation app, or the AIVA app for generating music. After reviewing important terminology in the generative AI field, he presented its underlying algorithms, as well as several of its applications and systems. Through several examples of current applications and tools, Professor Devedžić illustrated how to work with them in practice.

Research Associate Professor Dr. Biljana Stanković, coworker of **Research Professor Dr. Sonja Pavlović,** member of AMS-SMS, presented the use of machine learning in the detection of inflammatory bowel disease molecular biomarkers in her lecture titled "Application of artificial intelligence in the analysis of genetic data obtained by new-generation sequencing." She introduced the auditorium to the problem brought by modern high-throughput technologies such as next-generation sequencing that generate large amounts of biological data. The use of such extensive data requires advanced

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Figure 1. Opening address by Prof. Miloš Nedeljković, President of the Academy of Engineering Sciences of Serbia (AESS) and Past Dean of the Faculty of Mechanical Engineering, University of Belgrade (FMEUB) at the symposium titled "Artificial Intelligence and Medicine" – joint symposium of the Academy of Medical Sciences of the Serbian Medical Society and the AESS held on May 15, 2024 (Ceremonial Hall, FMEUB, Belgrade, Serbia); courtesy of FMEUB, Ivana Subašić

bioinformatics and statistical methods. In the presented study, the authors used human genome data obtained by the next-generation sequencing method, as well as data from medical records, and by machine learning algorithms selected molecular biomarkers that more accurately predicted the onset and the course of inflammatory bowel disease. These biomarkers significantly help in the prevention, early detection, and treatment of this disease.

Two lectures, one by an electrical engineer and the other by a doctor, dealt with the application of AI methods in medical imaging.

Professor Dr. Ana Gavrovska (School of Electrical Engineering, University of Belgrade) in her lecture titled "Modern automated approaches in improving medical imaging with the aim of effective diagnostics, management and risk assessment" pointed out that automated approaches based on AI can significantly improve medical imaging. Research in the field of medical imaging includes image quality improvements, detection performance enhancement, advanced image classification, estimation of possible user states, etc. All this makes medical image-based methods more effective, personalized, accessible, and less expensive. While presenting the modern achievements and possibilities of machine learning in working with medical images, Professor Gavrovska referred to current research at the Laboratory for Image Processing, Telemedicine and Multimedia, School of Electrical Engineering.

Professor Dr. Ružica Maksimović (Faculty of Medicine, University of Belgrade) began her lecture titled "Artificial intelligence in radiology" by asserting that AI has significantly contributed to improving the quality of diagnosis in various areas of radiology. However, she emphasized that radiologists should understand not only the value but also the pitfalls, weaknesses, and potential errors that may occur with the application of AI. Although AI algorithms are powerful, interpretation can lead to reduced diagnostic accuracy if tasked outside of its scope. This requires continuous training of radiologists and their close cooperation with software engineers and data scientists, because only such collaboration enables the correct choice of data analysis and processing methods to be used. This leads to reliable conclusions of key importance for the diagnosis and treatment of patients.

Ilija Tanasković, Master of Engineering (School of Electrical Engineering, University of Belgrade; The Institute for Artificial Intelligence Research and Development of Serbia) in his lecture titled "Application of artificial intelligence in the analysis of biomedical signals and images" introduced the audience to a large number of available trained models on large datasets that can be tailored to specific domains. Fine-tuning of the You Only Look Once (YOLO) model proved to be successful in the analysis of CT scans of patients with kidney tumors, enabling better detection and segmentation of the kidney tumor region (precision = 0.93, recall = 0.9). Analysis of biomedical electrocardiogram (ECG) signals using 12-channel ECG recordings showed success (93% area under the curve) for differentiating the normal signal from the signal of patients with myocardial hypertrophy, myocardial infarction, and ST/T changes. Additionally, ECG in combination with impedance cardiogram showed high accuracy in biometric identification.

Associate Professor Dr. Predrag Tadić (School of Electrical Engineering, University of Belgrade) held the lecture titled "Diagnosis of heart failure using machine and deep learning" co-authored by **Research Professor Dr. Jovana Petrović (**Vinča Institute of Nuclear Sciences, University of Belgrade). The lecturer first explained basic terms in the field of AI (expert system, machine/deep learning), and the principles on which their successful applications are based. Examples of successful applications of these techniques in medicine were then presented. Special attention was given to the SensSmart project funded by the Science Fund of the Republic of Serbia, whose goal is the development of a multi-sensor polycardiograph (stethoscope, ECG, accelerometer, photoplethysmography) and the accompanying algorithm based on machine and deep learning for the early diagnosis of heart failure. It was stated that within the SensSmart project, a database of recordings of sick and healthy subjects will be collected and made publicly available. This will contribute to solving one of the biggest obstacles in the wider application of machine learning in medicine, which the lack is of adequately annotated and publicly available medical data.

Professor Dr. Vladimir Mladenović (Faculty of Technical Sciences in Čačak, University of Kragujevac) presented in the lecture titled "Application of neural network models in predicting volume load in children on hemodialysis: an example of a case study of the application of artificial intelligence in medicine," the results of research conducted in cooperation with associate member of AMS-SMS **Professor Dr. Mirjana Kostić** (Faculty of Medicine, University of Belgrade) and their collaborators. Artificial neural networks were used in the study to obtain precise information about overhydration in children on regular hemodialysis. The obtained data on overhydration allows for the adjustment of dialysis parameters and the reduction of potential risks caused by excessive hydration. The example of the application of AI in hemodialysis illustrates how these technologies can improve the precision and efficiency of medical procedures, which contributes to improving the quality of life and patient outcomes.

Dr. Marina Popović Krneta (Faculty of Medicine, University of Belgrade)**,** coworker of **Professor Dr. Dragana Šobić Šaranović**, full member of AMS-SMS, in the lecture titled "Fundamentals of supervised machine learning and their practical application in nuclear endocrinology" presented their experience with the application of machine learning in order to identify predictive factors for the diagnosis and treatment of patients with papillary thyroid carcinoma (PTC). Through the examples of patients with PTC, predictive models based on different types of supervised machine learning were considered, which would allow for increased effectiveness of applied therapy in high-risk patients with PTC. On the other hand, it would enable avoiding unnecessary therapeutic protocols in patients where there is no clinical benefit from their application. In addition, she presented how the application of explainable machine learning methods can improve the interpretation and reliability of predictive models in medical research.

Assistant Professor Dr. Sc. Marija Živković (Faculty of Dental Medicine, University of Belgrade) held the lecture titled "Artificial intelligence in dentistry: opportunities and challenges." Firstly, she briefly presented the benefits of digital dentistry, which made a perfect introduction to the application of AI in dentistry. AI methods enable image analysis, more efficient treatment planning, and prediction of treatment outcomes due to the possibility of analyzing a large amount of data. Specifically mentioned topics included the importance of AI in the field of orthodontics. AI is characterized by high precision in determining cephalometric points on X-ray images with the help of convolutional neural networks, assessing whether extractions are needed within orthodontic therapy, as well as determining skeletal maturity based on the cervical vertebral maturation method. Although AI algorithms can significantly help in the interpretation of complex data, the final decision on the course of the treatment should still be made by the clinician, based on the available data and their personal experience.

Professor Dr. Jelena Roganović (Faculty of Dental Medicine, University of Belgrade) held the lecture titled "Ethical application of artificial intelligence in dental practice" that rounded off the content of the symposium, since the application of AI opens up numerous ethical challenges. The application of AI in dentistry requires that two questions are always considered: first – if and when AI tool should be applied; and second – how to engage AI in clinical decision-making. Furthermore, dentists need to acquire specific and AI-use-related skills in order to apply AI safely and effectively to dental patients. The results of the recent survey conducted at the Faculty of Dental Medicine in Belgrade showed that undergraduates compared to postgraduate dental students were skeptic about whether they should use AI in the practice at all. This could be a consequence of the lack of basic and continuing education regarding this subject, as well as of fear of risk that AI will replace dentists. Professor Roganović emphasized that the rapid development and introduction of AI methods into dental practices requires equally rapid preparation of legal and ethical regulations on the application of AI.

In the discussions after the individual lectures, symposium participants expressed their interest in certain presented methods as well as in the possibility of their application in practice. They also shared their experience in the application of AI and gave interesting suggestions for further research and cooperation. Ethical issues related to AI were mentioned several times in lectures and discussions, especially the necessity of legal and ethical regulations on the application of AI both globally and in our country.

The first joint meeting between AMS-SMS and AESS held in 2021 aimed to show the wide range of fields in which engineers and doctors collaborate [1]. The symposium titled "Artificial intelligence in medicine" was the first thematic symposium and it showed that AI is successfully applied in health and scientific institutions in Serbia thanks to the cooperation of engineers and doctors. AI has found applications in various branches of medicine,

so it is proposed to organize scientific meetings on the application of AI in specific branches of medicine in the future. This would contribute to the exchange of experience and knowledge, to better cooperation between teams of different professions, and to the further progress of both engineering and medicine.

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Ethics: This article was written in accordance with the ethical standards of the institutions and the journal.

Conflict of interest: None declared.

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Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонораришу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови**,** наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°*C*), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
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