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Erratum

In the double issue of the journal ***Medical Review*** 1 - 2/2023, on page 49 in the case report titled:
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there was an inadvertent error – the name of one co-author, Dr. **Dragan VELJOVIĆ**, was omitted.

Editorial Board

Erratum

U dvobroju 1-2/2023 na strani 49, rubrika Prikazi slučajeva u radu pod naslovom:
GIANT BREAST HAMARTOMA IN A MIDDLE-AGED WOMAN - A CASE REPORT
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došlo je do nenamerne greške – ispušten je jedan koautor dr **Dragan VELJOVIĆ**.

Uredništvo časopisa

PROFESSIONAL ARTICLES
STRUČNI ČLANCI

Academy of Vocational Studies Belgrade, Belgrade[1]
Health Center "Dr. Milutin Ivković" Palilula, Belgrade[2]
Secondary Medical School "Dr. Miša Pantić", Valjevo[3]

Professional article
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ANALYSIS OF CHANGES IN PHYSICAL ACTIVITY IN THE ADOLESCENT POPULATION DURING THE CORONAVIRUS DISEASE 2019 PANDEMIC

ANALIZA PROMENA U FIZIČKOJ AKTIVNOSTI U POPULACIJI ADOLESCENATA U TOKU PANDEMIJE COVID-19

Igor DIMITRIJEV[1], Ana LACKOVIĆ[2], Danijela PECARSKI[1], Nikola SAVIĆ[3] and Slavica ĐORĐEVIĆ[1]

Summary

Introduction. Physical activity affects all three domains of adolescent health. It affects physical, psychological and social functioning. During the period of the coronavirus disease 2019 pandemic, physical activity has changed significantly, which may affect the health potential of this population. **Material and Methods.** The research was conducted as a cross-sectional study. A questionnaire was designed as a research instrument in order to examine the personal experiences of adolescents in regard to physical activity during the coronavirus pandemic. The study included a sample of 120 students attending the Secondary Medical School "Dr Miša Pantić" in Valjevo. Participation in the study was voluntary and anonymous. **Results.** The largest number of respondents in the observed sample (N = 120) recognized that physical inactivity is one of the most significant risk factors for the health potential of adolescents. There is a statistically significant difference in the responses of the respondents from the aspect of engaging in physical activities during the coronavirus disease 2019 pandemic (p = 0.001). Respondents of both genders understand the importance of regular physical activity and its impact on mental health, sleep and rest, nutrition and other predictors that have a dominant influence on health. **Conclusion.** The commendable result is that adolescents recognize that physical inactivity is one of the greatest health risks. In preventive medicine, it is necessary to intensify health education programs that encourage the importance of physical activity in the period of adolescence.

Key words: Exercise; Habits; Adolescent; COVID-19; Pandemics; Risk Factors

Sažetak

Uvod. Fizička aktivnost utiče na sve tri determinante zdravlja adolescenata. Utiče na fizičko, psihičko i socijalno funkcionisanje. Tokom perioda pandemije COVID-19 fizička aktivnost je značajno izmenjena, što može uticati na zdravstveni potencijal ove populacije. **Materijal i metode.** Istraživanje je sprovedeno u formi studije preseka. Kao istraživački instrument konstruisan je upitnik koji ispituje lična iskustva adolescenata u vezi sa fizičkom aktivnošću tokom pandemije virusa korona. Istraživanjem je obuhvaćen uzorak od 120 učenika, koje je sprovedeno u Medicinskoj školi „Dr Miša Pantić" u Valjevu. Učešće u istraživanju bilo je dobrovoljno i anonimno. **Rezultati.** Najveći broj ispitanika u posmatranom uzorku (N = 120), prepoznaje da je fizička neaktivnost jedan od najznačajnijih faktora rizika po zdravstveni potencijal adolescenata. Postoji statistički značajna razlika u odgovorima ispitanika sa aspekta bavljenja fizičkim aktivnostima u toku pandemije COVID-19 (p = 0,001), ispitanici oba pola shvataju važnost kontinuirane fizičke aktivnosti i njenog uticaja na mentalno zdravlje, san i odmor, ishranu i druge prediktore koji dominantno utiču na zdravlje. **Zaključak.** Treba pohvaliti to što adolescenti prepoznaju da je fizička neaktivnost jedan od najvećih zdravstvenih rizika. U preventivnoj medicini potrebno je intenzivirati zdravstveno-vaspitne programe koji podstiču značaj fizičke aktivnosti u periodu adolescencije.

Ključne reči: fizička aktivnost; navike; adolescenti; COVID-19; pandemije; faktori rizika

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has caused disruptions in the global social system. Adolescents faced social role changes, the schools were closed, activities that can be carried out outside the home were restricted, and many contacts with peers in the free time were reduced [1]. Numerous studies have found that physical activity affects all three domains of health. It has a great impact on the mental health of adolescents during the pandemic. The impact of psychologically stressful situations

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Abbreviations
COVID-19 – coronavirus disease 2019
ANOVA – analysis of variance

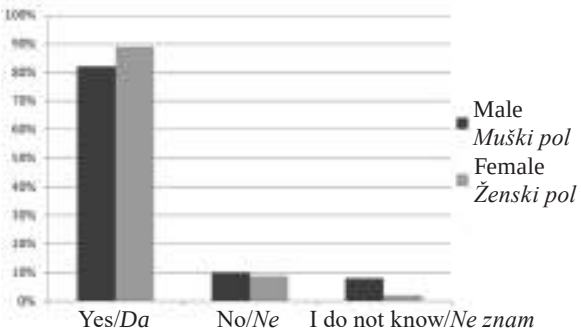
such as the COVID-19 pandemic on adolescents is a health risk that may lead young people to serious health problems. The consequences of physical inactivity and interrupted social pattern of behavior can be very unfavorable for adolescents [1, 2]. Numerous scientific studies testify to the decline in sports activities among adolescents during the corona virus pandemic. Physical activity in a large number of cases requires social interaction and collective sports were not always available; this resulted in an increase in the number of hours that adolescents spent in front of a computer or phone screen [3]. The negative impact of physical inactivity has also led to an increased number of obese children. Although obesity was a big problem among adolescents before the pandemic as well, physical inactivity has worsened this problem, but there are still no final results on how nutrition and physical inactivity affected adolescents. Obesity is something that accompanies physically inactive people and it causes numerous metabolic, cardiovascular and other health disorders and diseases [4, 5]. Physical inactivity negatively affects the circadian rhythm, sleep and rest [6]. Numerous studies showed that inadequate sleep, altered activities of daily life, online teaching from home during the COVID-19 pandemic negatively affected health-related habits in adolescents. During this period, young people were much less physically active, stayed in front of the screen longer in the evening and had an irregular sleep/wake rhythm [7]. A great number of scientific studies that have dealt with the physical inactivity of adolescents during the COVID-19 pandemic have proven the connection between this health risk and many physical and psychological health problems. Physical exercises at home, training with a specific program and social interaction are associated with better health outcomes during the coronavirus pandemic [8]. The physical inactivity of adolescents during the quarantine and state of emergency may lead to the adoption of negative behavior patterns, which will continue even after the conditions imposed by the pandemic change. Physical activity is also influenced by the habits that young people had before the pandemic [9]. Physical activity, moderate and dosed, during the pandemic enabled a lower impact of numerous stressors faced by adolescents in this period [10]. Isolation and restriction of people's movement is a public health measure that has saved many lives, yet it leaves consequences in terms of reduced physical activity of adolescents, so strategies are needed to help the implementation of preventive measures to alleviate negative lifestyle habits in adolescents [11]. A large number of scientific studies point to the decline of physical activity, primarily in the population of men compared to women, dominantly reduced intense physical activities and training, followed by walks and moderate physical activities aimed at relaxation [12]. Long COVID-19 measures can lead to long-term barriers in engaging in physical activities, the feeling of exhaustion and numerous health problems can negatively affect proper physical activity and the health potential of an individual. These problems are rarer in the adolescent population, but they are not impossible, thus additional health education support is needed by experts in the field of preventive medicine [13]. The reduction of physical activity during the pandemic resulted in the development of other health risks, such as the consumption of alcohol, cigarettes and psychoactive substances, which can permanently damage the health potential of adolescents [14]. Physical activity is effective in overcoming mental problems, anxiety and depression, prevention of obesity and other health risks; it should be applied daily and adapted to the life situation [15, 16].

Material and Methods

The research was conducted as a cross-sectional study. A questionnaire was designed as a research instrument to assess the habits of adolescents regard-

Table 1. Sociodemographic characteristics of respondents
Tabela 1. *Sociodemografske karakteristike ispitanika*

Distribution criteria *Kriterijum distribucije*	Variables *Varijable*	No *Apsolutni broj*	% *Procenat*
Gender *Pol*	Male/*Muški*	43	36%
	Female/*Ženski*	77	64%
Place of living *Mesto stanovanja*	Urban environment/*Urbana sredina*	65	54%
	Suburban settlements/*Prigradska sredina*	12	10%
	Rural areas/*Ruralna sredina*	43	36%
Maternal education *Obrazovanje majke*	Primary school/*Osnovna škola*	9	8%
	High School/*Srednja škola*	84	69%
	University education/*Univerzitetsko obrazovanje*	27	23%
Paternal education *Obrazovanje oca*	Primary school/*Osnovna škola*	8	7%
	High School/*Srednja škola*	81	68%
	University education/*Univerzitetsko obrazovanje*	31	25%



Graph 1. Is physical inactivity one of the most serious risk factors for health?
***Grafikon 1**. Da li je fizička neaktivnost jedan od najozbiljnijih rizika po naše zdravlje?*

ing their physical activities during the COVID-19 pandemic. It included a sample of 120 adolescents attending the Secondary Medical School "Dr. Miša Pantić" in Valjevo, in the period from March to May 2021. The sample was intentional, including students of the final grades of medical profiles. Participation in the study was voluntary and anonymous and the research was approved by the competent authorities of the Secondary Medical School "Dr Miša Pantić" Valjevo. Before the beginning of the research, the respondents received necessary information on the study orally and in writing and they submitted their written consent for the survey. The students were given instructions on how to complete the questionnaire. The researchers personally distributed the questionnaires and collected the data. Statistical data processing included methods of descriptive and inferential statistics using the analysis of variance (ANOVA) test. Data were analyzed using the Statistical Package for the Social Sciences for Windows. The obtained results are presented in tables and graphs.

Results

The most important sociodemographic characteristics of the respondents in the observed sample are shown in **Table 1**.

The largest number of respondents of both sexes believes that physical inactivity is one of the greatest risk factors for the health potential of adolescents **(Graph 1)**.

Table 2. Personal experiences of adolescents related to physical activities during the COVID-19 pandemic
***Tabela 2.** Lična iskustva adolescenata u vezi sa fizičkim aktivnostima u toku pandemije COVID-19*

Variables *Varijable*	Likert scale - mean value *Likertova skala – srednja vrednost*		ANOVA	p/*p*
	Mean - Male *Sr. vred. Muški pol*	Mean - Female *Sr. vred. Ženski pol*		
Physical activity longer than 60 minutes per day *Fizička aktivnost duža od 60 minuta na dan*	2.42	2.11	10.253	**0.001**
Do you do sports regularly?/*Da li se redovno bavite sportom?*	1.10	1.09	25.241	**0.001**
Moderate physical activity during the day *Umerena fizička aktivnosti u toku dana*	1.10	1.09	25.241	**0.002**
Did you exercise at home during the COVID-19 pandemic? *Da li ste se bavili fizičkim aktivnostima u svom domu tokom pandemije COVID-19?*	2.11	1.60	25.120	**0.002**
Has the COVID-19 pandemic affected your physical activity habits?/*Da li je pandemija COVID-19 uticala na Vaše navike u vezi sa fizičkim aktivnostima?*	3.14	2.39	94.578	**0.001**
Do physical activities have a positive effect on your mental and physical health?/*Da li fizička aktivnost ima pozitivan efekat na Vaše mentalno i fizičko zdravlje?*	3.62	3.83	7.354	**0.004**
Did physical activity help you overcome feelings of anxiety and depression during the corona virus pandemic? *Da li vam je fizička aktivnost pomogla u prevazilaženju osećaja anksioznosti i depresije u toku pandemije korona virusa?*	3.07	3.41	17.981	**0.001**
Is physical activity beneficial for sleep and rest? *Da li fizička aktivnost korisna za san i odmor?*	2.92	3.65	29.189	**0.001**
Is your diet healthy and balanced in relation to your physical activities?/*Da li se trudite da se hranite zdravo i balansirano u odnosu na fizičku aktivnost?*	3.52	2.68	42.623	**0.004**
Is it necessary for adolescents to engage in physical activities and sports even during the pandemic?/*Da li je neophodno da se adolescenti bave fizičkim aktivnostima i sportom i tokom pandemije?*	2.60	1.65	45.692	**0.002**
Is moderate physical activity recommended for everyone? *Da li se umerena fizička aktivnost preporučuje svima?*	1.64	2.24	0.344	0.228

Discussion

A total of 120 respondents participated in the research related to the physical activity of adolescents during the COVID-19 pandemic **(Table 2)**. The sample included 77 (64%) female and 43 (36%) male respondents. Most respondents in the study were from urban areas (54%), rural areas (43%) and the smallest number of respondents was from suburban settlements (12%). Regarding the parental education, the dominant educational level was high school (69% of mothers and 68% of fathers). Most respondents engaged in physical activities every day, even during the pandemic ($p = 0.001$). There was a statistically significant difference between respondents in regard to regular physical activity; sports were more dominantly played by male respondents ($p = 0.001$). Subjects of both sexes had moderate physical activity during daily activities. There was a statistically significant difference in regard to physical activities and sports during the COVID-19 pandemic at home; these were also more dominant in male respondents ($p = 0.002$). Male respondents also confirmed that the COVID-19 pandemic affected their physical activity habits ($p = 0.001$). There was a statistically significant difference regarding positive effects of physical activity on the mental health; positive responses predominantly came from female respondents ($p = 0.004$). In the observed sample of respondents, female adolescent claimed that physical activity during the coronavirus pandemic helped them a lot in overcoming feelings of depression, stress and anxiety. Respondents of both sexes agreed that physical activity helps regulate sleep and rest (0.001). When it comes to proper nutrition and physical activities, male respondents took more care about proper nutrition ($p = 0.004$). There was a statistically significant difference between respondents regarding the necessity to maintain habits related to physical activity even during the pandemic ($p = 0.002$). A small number of adolescents recognized that everyone needs moderate physical activity during the day (0.228). The largest number of respondents in the observed sample claimed that physical inactivity is one of the most dangerous health risks for adolescent health; 82% of male respondents and 89% of female respondents. The results of our study match the results of other researchers in eminent databases. Researchers from the University of Edinburgh also obtained results that indicate the impact of physical activity on body image, nutrition and mental status [17]. Researchers from China also reported that social conditions caused by the pandemic affected the health potential of adolescents through social distancing, closing schools, restricting group sports activities that negatively affected the physical activity of adolescents, which coincides with our results [18]. The results of researchers from the Ludwig-Maximilians-University Hospital, Munich, Germany, are in agreement with our results; diet and physical activity are important predictors of health in both pandemic and emergency situations. All this affects the physical and psychological health of adolescents [19]. Researchers from Austria agree that the COVID-19 pandemic had a particularly stressful effect on younger people, people with low incomes and adolescents [20].

Conclusion

Physical activity affects all three domains of adolescent health. It affects physical, psychological and social functioning. During the period of the coronavirus disease 2019 pandemic, physical activity has changed significantly, which may affect the health potential of the adolescent population. Physical inactivity may cause irreversible damage to health and development of comorbidities in adolescents. Preventive interventions by healthcare workers should be more intensive in regular circumstances but also in situations such as a pandemic.

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INNER EAR, NOSE AND PHARYNX – SIGNS AND SYMPTOMS OF CORONAVIRUS DISEASE 2019 INFECTION

UNUTRAŠNJE UVO, NOS, ŽDRELO – SIMPTOMI I ZNACI BOLESTI COVID-19

Milica PISARIĆ[1] and Slobodanka LEMAJIĆ KOMAZEC[1, 2]

Summary

Introduction. The most common clinical symptoms of coronavirus disease 2019 include cough, high body temperature, malaise, weakness, headache, and diarrhea. When the pandemic slowed down, more and more patients reported symptoms atypical for the infection, such as hearing loss, tinnitus, and vertigo. The aim of this study was to assess current knowledge and data on the existence and prevalence of otorhinolaryngology sings and symptoms of coronavirus disease 2019. **Material and Methods**. The literature was reviewed from May to December 2022 and included articles published in 2020 or later. The main criterion was confirmed severe acute respiratory syndrome coronavirus 2 infection by means of the polymerase chain reaction test. **Results**. Sensorineural hearing loss presents as acute, chronic and subclinical. Tinnitus is associated with direct viral invasion and social factors. Vertigo is also associated with direct viral invasion and prolonged bed rest. Olfactory and gustatory disorders are known symptoms of viral infections. Olfactory dysfunction occurs as a consequence of the existence of angiotensin-converting enzyme 2 receptors in the nasal mucosa, which is the primary site for viral binding, and which explains the absence of nasal congestion. It has been shown that the pediatric population presents with different clinical symptoms of the infection. In children, rhinorrhea and pharyngitis are the most common symptoms, while in adults they are generally absent. **Conclusion**. The available literature data showed that otorhinolaryngology symptomatology of coronavirus disease 2019 is present, but the data are still very limited. The literature showed vast discrepancies in the prevalence and risk factors associated with coronavirus disease 2019. It is imperative that more research is done on the topic now that the pandemic is subsiding, and more attention should be paid to non-life-threatening symptoms.

Key words: COVID-19; Signs and Symptoms; Labyrinth Diseases; Nose Diseases; Pharyngeal Diseases; Hearing Loss; Vertigo; Tinnitus; Audiology; Otolaryngology

Sažetak

Uvod. Uobičajena klinička slika COVID-19 oboljenja se sastoji iz kašlja, visoke telesne temperature, slabosti, malaksalosti, glavobolje i dijareje. Kada se usporio talas pandemije, sve više pacijenata je prijavilo simptome koji su bili netipični za infekciju. Ti simptomi su bili gubitak i oštećenje sluha, tinitus, vertigo. Cilj ovog rada bio je da se proceni trenutno dostupno znanje i podaci o postojanju i prevalencije otorinolaringoloških simptoma COVID-19 infekcije. **Materijal i metode**. Literatura je pregledana od maja do decembra 2022. godine. Literatura je morala da bude objavljena najkasnije 2020. godine. Glavni kriterijum je bila potvrda postojanja SARS-CoV-2 infekcije putem testa lančane reakcije polimeraze. **Rezultati**. Senszorineuralno oštećenje sluha je prijavljeno kao akutno, hronično i supkliničko. Tinitus je povezan sa direktnom virusnom invazijom i socijalnim faktorom. Vertigo je takođe povezan sa direktnom virusnom invazijom ali i produženim ležanjem. Olfaktorna i gustatorna disfunkcija su poznati simptomi virusnih infekcija. Olfaktorna disfunkcija nastaje kao posledica postojanja angiotenzin-konvertujućeg enzimskog receptora u nosnoj sluzokoži za koje se vezuje virus, što objašnjava nedostatk nazalne kongestije. Pokazalo se da pedijatrijska populacija ima drugačiju kliničku sliku. Kod dece, rinoreja i faringitis predstavljaju neke od najčešćih simptoma dok se kod odraslih izuzetno retko javljaju. **Zaključak**. Podaci dostupni u sadašnjoj literaturi nam pokazuju da je otorinolaringološka simptomatologija COVID-19 infekcije prisutna ali su podaci i dalje veoma oskudni. Literatura pokazuje velike diskrepance kod prevalencije i faktora rizika povezanim sa COVID-19 oboljenjem. Imperativ je da se dodatna istraživanja urade na datu temu sada kada pandemija jenjava i kada se više pažnje može posvetiti životno neugrožavajućim simptomima.

Ključne reči: COVID-19; znaci i simptomi; bolesti unutrašnjeg uha; oboljenja nosa; oboljenja ždrela; gubitak sluha; vrtoglavica; zujanje u ušima; audiologija; otorinolaringologija

Introduction

Coronaviruses are ribonucleic acid (RNA) viruses that cause diseases in mammals and birds. In mammals, they commonly cause respiratory tract infections with symptoms like malaise, general weakness and headache and gastrointestinal symptoms, mostly diarrhea. As the number of coronaviruses rises, they are classified into 4 genera: alpha, beta, delta, and gamma. Currently, 45 types of coronaviruses are known, out of which 7 that infect humans have been identified. Common human coronaviruses include types 229E, OC43, NL63, HKU1, but they are also the least known since they usually do not cause symptoms in immunocompetent people. If they do cause symptoms, they are usually flu-like symptoms. Severe acute respiratory

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Abbreviations

COVID-19	– coronavirus disease 2019
SARS-CoV-2	– severe acute respiratory syndrome coronavirus 2
PCR	– polymerase chain reaction
ACE2	– angiotensin-converting enzyme 2
RNA	– ribonucleic acid
ENT	– ear, nose, throat
MRI	– magnetic resonance imaging
TEOAE	– transient evoked otoacoustic emission

syndrome coronavirus 2 (SARS-CoV) and Middle East respiratory syndrome coronavirus are viruses of animal origin and are linked to epidemics in 2002 and 2012. If we take into account big viral genomes, a lot of genetic variants, frequent recombination within the viral genetic material, wide distribution and ever more frequent interactions between humans and animals, we can assume that epidemics and pandemics like these will periodically be active [1].

The COVID-19 is a disease caused by SARS-CoV-2, which is the newest type in the Coronaviridae family, and it is the seventh type of beta coronavirus. It is a zoonosis; the virus is transmitted via droplets from respiratory tract of infected persons, either directly to the exposed individual, or indirectly to exposed surfaces which healthy individual will come in contact with. The infected persons are usually contagious two days before the symptoms appear and in the first few days of illness. People who develop a severe clinical presentation can be infections even longer. Also, asymptomatic cases are a major problem, because they may disseminate the virus unknowingly. Clinical symptoms usually develop 4 to 5 days after exposure to the virus [1].

The S protein, which binds to angiotensin-converting enzyme 2 (ACE2) receptor, is the most important for the pathogenesis of the virus, while ACE2 receptors are found in many tissues of the human body which explains the broad spectrum of symptoms. Factors such as older age, male sex, ethnicity, high body mass index, hypertension, obesity, immunosuppressants, diabetes, and cardiovascular diseases cause higher expression of ACE2 receptors which may lead to a more severe clinical presentation [2].

When the virus comes in contact with the host's cells after binding to ACE2 receptors, viral RNA enters the cells, it replicates and infects the surrounding cells and this process repeats until it reaches alveoli. Alveolar destruction causes loss of vascular integrity and increased capillary permeability. This leads to pulmonary edema, hypoxia, and progressive lung damage. After local destruction, virus enters the bloodstream and disseminates through the organism. When the immune system is activated, "cytokine storm" leads to hypotension and acute respiratory distress syndrome [3].

As mentioned above, symptoms of virus infection include cough, high body temperature, malaise, weakness, headache, and diarrhea. On the other hand, when the first pandemic wave slowed down, more and more people reported some manifestations that differ from usual presentation, including hearing loss, tinnitus, vertigo and other symptoms that are in the domain of otorhinolaryngology [1, 4].

Symptoms that were linked to COVID-19 by general public, loss of smell and taste, are ear, nose, and throat (ENT) symptoms. There is a wide range of ENT symptoms that have been reported with different prevalence and risk factors in a number of studies. Symptoms that have been reported are sensorineural hearing loss, deafness, tinnitus, vestibular disorders, olfactory and gustatory dysfunction, pharyngitis, tonsillitis, and rhinorrhea [4].

Some of the pathophysiological mechanisms that are speculated to be the cause of auditory and vestibular symptoms are immunological, vascular and cellular. The virus is neurotrophic and neuroinvasive and it damages the cochlear nerve leading to hearing loss, deafness, and balance disorders. Besides the mentioned mechanism, the ACE2 receptors are expressed in the temporal lobe and cerebellum, which can explain how the virus links to hearing and balance part and of the brain. Presence of virus in the bloodstream causes a prothrombotic state which leads to production of small thrombi that mainly affect small vessels, such as vessels of the cochlea that leads to ischemia and distress of the inner ear receptors. In addition to mentioned mechanisms, it also leads to inflammation that causes release of cytokines which has negative impact on the inner ear cells [3–5, 7].

Tinnitus is explained by the above mentioned mechanisms with an addition of social factors. Tinnitus is a condition that is easily aggravated with stress, meaning that the pandemic as a factor itself cannot be excluded. New onset or worsening of tinnitus may have a dual etiology: above explained mechanisms where SARS-CoV-2 leads to metabolism changes, and stress from being in quarantine, social isolation, and depression with no direct link with the virus itself [8, 9].

Olfactory dysfunction is not new to medicine when it comes to viral infections, but it is a pathophysiological mechanism that is different in COVID-19. Loss of smell and taste has previously been linked to nasal obstruction and rhinorrhea. However, since COVID-19 leads to nasal obstruction and rhinorrhea in small percentage of cases, it is speculated that SARS-CoV-2 leads directly to the damage of mucosa and it invades the nervous system that participates in smell and taste functions. Invasion of the olfactory bulb is found on magnetic resonance imaging (MRI) of the infected patients [8, 10, 11].

Rhinorrhea is a common symptom of viral infections in general, but it is uncommon as a COVID-19 manifestation. Reasons for the lack of this symptom are still unknown [3].

Pharyngitis and tonsillitis are caused by direct viral invasion of the mucosa, as it is the case with viral infections in general [3, 12].

The aim of this paper was to confirm that COVID-19 is not only a respiratory infection and that it affects the ENT region as well.

Hypotheses:

1. COVID-19 affects the ENT region;
2. COVID-19 can cause both short-term and long-term ENT consequences.

Material and Methods

This retrospective review included data bases such as PubMed, MEDLINE, Web of Science, and Google Scholar. The review was conducted from June to December 2022.

The key words used in the research were SARS-CoV-2, COVID-19, ENT, otorhinolaryngology, audiology, balance, hearing loss, deafness, vertigo, tinnitus, smell, taste, olfactory dysfunction, gustatory dysfunction, rhinorrhea, anosmia, pharyngitis, and tonsillitis.

Confirmed COVID-19 infection by means of PCR was the main inclusion criterion. Studies and case reviews without COVID-19 positive PCR were not included. All reviewed papers were in English. The study included articles published in 2020 or later.

Results

Sensorineural hearing loss

The first case study mentioning sensorineural hearing loss was by Sriwijitalai W. and Wiwanitkit from Thailand, where one female patient out of 82 came forward with a new-onset hearing loss which was confirmed by tone audiometry. It was reported that the patient received all necessary supportive and symptomatic treatment for her general condition, but it was not mentioned if she received any treatment specifically for hearing loss. Tone audiometry in post-infectious period did not show any improvement [13].

Degen C. et al. reported a patient (60-year-old male) who was in the state of hyperactive delirium after 13 days in the intensive care unit. After resolution of psychotic symptoms, the patient experienced deafness in the right and hearing loss in the left ear. Tone audiometry confirmed the findings and MRI was performed. It showed that an inflammatory process in the right cochlea may lead to ossification. Therefore, right ear cochlear implantation was performed before ossification occurred. The left ear was treated by intratympanic steroid injections. During hospitalization, the patient received azithromycin and furosemide, both ototoxic, but they were ruled out as the cause of hearing loss because they would have caused equal bilateral hearing loss without changes on the MRI [14].

Abdel Rahman S. and Abdul Wahid A. reported a 52-year-old male patient with a left-sided hearing loss and progressive tinnitus. He was positive for COVID-19, but showed no other symptoms except hearing loss. Tone audiometry confirmed it, but the MRI showed no changes. After 3 intratympanic steroid injections, the patient partially restored function [15].

Saniasiaya J. reported a 45-year-old male patient with asthma who required ventilation in the intensive care unit and who was intubated for 30 days. Later, the patient developed pulmonary hypertension, pulmonary embolism, ventilator acquired pneumonia, and anemia. His treatment included remdesivir and intravenous steroids. A week after extubation the patient developed tinnitus and hearing loss on the left side. Two days before the onset of cochlear symptoms, the patient received teicoplanin and ciprofloxacin. No known ototoxic drugs were administered. Hearing loss was confirmed with tone audiometry (threshold was 65, 75, 75, 85 dB on 2, 3, 4, 6 kHz, respectively) while MRI showed no changes. The patient received 60 mg of prednisolone orally, after which intratympanic administration was introduced. Only partial improvement was recorded (TA: 55, 60, 60, 80 dB on 2, 3, 4, 6 kHz, respectively) [6].

Kilic O. et al. conducted a research in Turkey including 5 patients who came to ENT Clinic with acute hearing loss as an isolated symptom. After hearing loss was confirmed, PCR was performed and one patient was positive. People with negative PCR were treated with 1mg/kg/day of prednisolone per os, vitamin B complex, folic acid and protein pump inhibitors, while the COVID-19 positive patient was treated with 200 mg of hydorxychloroquine twice a day. All patients restored hearing completely in 10 - 14 days, including the COVID-19 positive patient who was on antiviral drugs instead of steroids [16].

Mustafa M.W.M. conducted a study in Egypt comparing otoacoustic emissions and tone audiometry tests between asymptomatic COVID-19 positive patients and healthy people. The test group included 20 COVID-19 positive patients, while the control group included 20 healthy people (hearing threshold over 15 dB). To exclude age-related effects on the inner ear, patients were aged from 20 to 50 years. Two weeks after COVID-19 confirmation, otoacoustic emissions and tone audiometry were conducted in both test and control group. Statistically significant difference was found between two groups by tone audiometry at 4, 6 and 8 kHz ($p < 0.05$), as well as by otoacoustic emissions ($p < 0.001$). Tone audiometry and otoacoustic emissions were worse in the test group, even though people did not have any symptoms and their hearing loss was subclinical but very much real. Transient evoked otoacoustic emission (TEOAE) showed that there was damage to the outer hearing cells without clinically manifested hearing loss. This study also showed that even without severe clinical presentation, other organs, apart from the respiratory system, can be involved [17].

Saniasiaya researched a database in Malaysia and concluded that 35 patient aged 20 to 60 years met criteria for COVID-19 related hearing loss. All patients had a hearing loss (partial or complete) as the primary symptom, while secondary symptoms were tinnitus, otalgia, and vertigo. Tone audiometry, TEOAE and tympanometry were used to confirm the findings [6].

Fancello V. et al. used a questionnaire and concluded that the most common ENT symptom of SARS-CoV-2 infection is sensorineural hearing loss that occurred in 15 out of 20 patients with ENT symptoms. Out of 15 patients with hearing loss, 5 had isolated hearing loss, 7 had hearing loss and tinnitus, while 3 patients had hearing loss, tinnitus and vertigo. In this study, they reported that 23.2% out of 185 patients had tinnitus after COVID-19 confirmation. The negative side of this questionnaire is inability to objectively measure tinnitus and possible involvement of other factors in tinnitus development (pandemic, isolation, depression, anxiety) [18].

Tinnitus

Beukes E. et al. performed a meta-analysis on tinnitus using the available literature published up to March 21, 2021. Their focus was on researching the impact of virus itself on tinnitus and the impact of pandemic on tinnitus. Their analysis included 35 patients with auditory symptoms aged 23 to 67 (mean age 42). Out of 35 patients, 14 had tinnitus (40%), 6 were male (43%) and 8 female (57%). This study showed no correspondence in location of tinnitus. Also, there was no consistency regarding the time of tinnitus onset and COVID-19 diagnosis. Some of the studies they included in their analysis, reported tinnitus onset with COVID-19 diagnosis, one reported tinnitus onset after 13 days in intensive care unit, while one study reported tinnitus onset a week after leaving the intensive care unit. Only 2 patients reported complete resolution of tinnitus while others reported tinnitus even in the post infectious period [19].

Vertigo

Maslovara S. and Košec A. reported on two similar case studies. Two female patients (41 and 28 years old) developed short, intensive vertigo with nausea and vomiting more than a month after getting negative COVID-19 tests after two weeks of self-isolation and being COVID-19 positive. They both denied having any other symptoms. Dix-Hallpike test showed canalithiasis of the right posterior semicircular canal in both cases. Also, in both cases after 2 Epley maneuvers, both patients confirmed resolution of vertigo [20].

These two cases turn our focus to vestibular symptomatology that viral infection can cause. It is important to include vestibular neuritis (that is usually of viral origin) and benign paroxysmal positional vertigo in differential diagnosis. Benign paroxysmal positional vertigo accounts for 17.1% of vertigo cases in general population, while in the geriatric population prevalence goes to 50% [20, 21].

Almishaal A. A. and Alrushaidan A. A. created a questionnaire that was completed by 301 patients. Out of 301 patients, 65.9% were not hospitalized while 35.22% (103) were admitted to the intensive care unit. Out of all patients, 69.1% (208) reported at least one comorbidity. In the acute phase of infection, 11.63% were asymptomatic, while others had more symptoms. Most common symptoms were cough, elevated body temperature, malaise, loss of taste and smell; 21.93% reported auditory symptoms out of which 19 (6.31%) reported hearing loss, 30 (9.97%) tinnitus, 57 (18.94%) fullness in their ear, 6 (1.99%) difficulties of conversation in quiet environment, 10 (3.32%) difficulties in noisy environment. Even after 6 months, 11 (3.65%) patients reported presence of symptoms in milder forms [22].

Furthermore, 105 patients (34.88%) experienced vestibular symptoms, out of which 25 (8.31%) reported instability, 90 (29.9%) nausea, and 73 (24.25%) vertigo. In comparison to patients with auditory symptoms, patients with vestibular presentation were younger (33.35 ± 10.98 years). After 6 months, 12 patients (3.99%) still experienced vestibular symptoms. The authors established that the greatest predictors of auditory and vestibular difficulties were tiredness and malaise [22].

Olfactory and gustatory dysfunctions

Lechien JR. et al. conducted a multicentric European study including 417 patients out of which 357 (85.6%) had olfactory symptoms. There were 284 (79.6%) patients with anosmia and 73 (20.4%) with hyposmia. These symptoms occurred before (11.8%), after (65.4%) and at the same time as other symptoms (22.8%). Out of 247 patients that experienced regression of other symptoms and had a negative COVID-19 test, olfactory dysfunction persisted in 63% of cases. The patients were contacted 15 days after resolution of symptoms, not after a couple of months [10].

Gustatory dysfunctions were present in 342 patients (88.8%). In 78.9% of patients, sense of taste was disrupted, and in 21.1% it was completely gone. The loss of smell and taste were constant during infection in 72.8%, while in 23.4% it fluctuated. Out of all patients who reported loss of smell and taste only 3.8% showed correlation with nasal congestion therefore showing that congestion is a secondary mechanism in development of these symptoms. Gustatory dysfunction was not reported 15 days after resolution of other symptoms [10].

Agyeman AA. et al. performed a literature review about olfactory and gustatory symptoms related to COVID-19 with the aim to confirm the prevalence of these disorders. The study included 8,438 patients in a variety of published papers. The mean age was from 34 to 77 years and 58.7% (4,785 from 8150) were females. Out of 24 studies about olfactory disorders, 21% used objective methods of assessment while others relied on patients' self-reports. The prevalence ranged from 3.2% to 98.3% [23].

Gustatory dysfunctions were analyzed including 15 studies and 5,649 patients. Only 13% of studies used objective methods of assessment (UPSIT test, CCCRC test, sniff sticks), while the rest relied on patients' self-reports. The prevalence ranged from 5.6% to 62.7% [23].

Rhinorrhea

When it comes to pediatric population, Neha A. Patel reported that in children symptomatology and prevalence were different. Her results showed that out of 2,914 pediatric patients, 56% were boys, 79% did not have any comorbidities. Out of 21% children with comorbidities mostly presented with asthma, immunosuppression, and cardiovascular diseases. The study showed that 14.9% were completely asymptomatic. The most common symptom was cough (48%), elevated body temperature (47%) and pharyngitis (28.6%). Rhinorrhea was reported only in 13.7%, nausea and vomiting in 7.8% and diarrhea in 10.1%. This study shows that children really present with milder clinical symptoms than adults. Furthermore, this study shows that rhinorrhea is more common in children (13.7%) than in adults (2%) [12].

Pharyngitis

El-Anwar MW. et al. collected available data and included 1,773 COVID-19 positive patients with ENT symptoms. They reported that rhinorrhea was present in 38 patients (2.1%), nasal congestion in 72 patients (4.1%), olfactory dysfunction in 107 (6%), pharyingitis in 200 (11.3%), pharyngeal erythema in 98 (5.3%), tonsillitis in 23 (1.3%), headache in 189 (10.7%) and upper respiratory tract symptoms in 33 patients (1.9%). Based on available data, the authors concluded that the most common sings of infections were elevated temperature (1,303 patients, 73.5%), dry cough (1,080, 61%), productive cough (405, 22.8%), dyspnea (288, 16.2%), hemoptysis (10, 0.6%), chest pain (2, 0.1%), nausea/vomiting (69, 3.9%), constipation (2, 0.1%), diarrhea (75, 4.2%), malaise (484, 27.2%), and myalgia/arthralgia (185, 10.4%). Based on their findings, the most common ENT symptom is pharyngitis (reported in 11.3% of cases) [4].

Zimmermann P. and Curtis N. performed a literature review and analyzed 333 pediatric patients. They reported that 35% of children were asymptomatic, 42% had elevated body temperature, and pharyngitis was found in 30% of cases. They concluded that these symptoms were the most common clinical findings in children. This shows that children and adults have different clinical presentations [24].

Additionally, children are more susceptible to anemia, which is one of the risk factors for lower respiratory tract infections. Apart from that, neutrophilia and both high and low lymphocyte counts are common in pediatric population and are not so frequent in adults [25].

Discussion

The SARS-CoV-2 enters the human body and then starts replicating in human cells. After the first contact with the virus, it replicates inside the nasopharynx and that circle repeats until it reaches alveoli. Inside the lungs, it continues to replicate which leads to destruction of pulmonary tissue. Lung damage is associated with a risk for development of acute respiratory distress syndrome and also for virus spreading throughout the organism. It is spreading binding to erythrocytes and later binding to every cell with ACE2 receptor. The ACE2 receptors are found in vast types of tissues in the human organism. Computed tomography and MRI confirm that the virus has a tropism for the nervous system and that it is neuroinvasive which explains the existence of auditory, vestibular, gustatory, and olfactory symptoms [2–4].

The ENT symptoms are manifestations of COVID-19, but there are still not enough studies that would lead to knowing the absolute truth. Reasons for that are multifactorial. Firstly, pandemic caught all of us by surprise leading to great influx of patients which overwhelmed health systems all around the world. Great number of patients and emergencies caused less time for medical history taking and physical examination. Also, a lot of patients required emergency treatment due to their respiratory status which pushed back all non life threatening symptoms. In addition to all above mentioned, all around the world there were problems about resources, medical staff and medical equipment. On the other side, every research required all equipment to be sterilized between each patient which was complicated and time and resource consuming. With all that said, it is understandable that non urgent symptoms were disregarded at the peak of the pandemic. In addition, at the time of writing this article, there was no worldwide consensus agreed upon treatment of COVID-19 patients. Steroid use during infection is also a great problem, since it weakens the immune system and changes the response of a human body to virus [1, 3].

Most researchers agree that the most common SARS-CoV-2 infection symptoms are elevated body temperature, diarrhea, cough, tiredness, malaise, myalgia, and arthralgia. Also, it is a fact that patients present with ENT symptoms after COVID-19. The existence of these symptoms points to neural tropism of SARS-CoV-2 in contrast to SARS-CoV and Middle East respiratory syndrome coronavirus [4].

There is no way to be certain about the risk factors for ENT manifestations and which symptom is the most common just yet. According to El-Anwar MW. et al., the most frequent ENT symptom is pharyngitis, while according to Fancello V, Hatzopoulos S, and Corazzi V, it is sensorineural hearing loss. Nevertheless, ENT manifestations of COVID-19 are real and reported. It is now time to globally agree on how to treat patients with COVID-19, then to document data regarding ENT symptoms with aim to get unanimous prevalence, risk factors, and most importantly, a cure for them [4, 19].

Thus, the hypotheses were confirmed:

3. COVID-19 affects the otorhinolaryngology region;

4. COVID-19 can cause both short-term and long-term otorhinolaryngology consequences.

The advantage of this paper is that it shows that there are still not enough data and knowledge about ENT symptoms of COVID-19 and it turns the spotlight on the missing data in hope that it will lead to more research.

The limitation of the paper is that, since there are still not enough data, studies from around different parts of the world were compared, which can mean that data differ, because there was no one accepted consensus when it comes to treatment of COVID-19 worldwide. There is a possibility that excessive antimicrobials and steroids were used, which may impact the function of ENT organs.

Conclusion

Severe acute respiratory syndrome coronavirus 2 is a new strain of coronavirus which causes primarily respiratory infections, but researchers have confirmed its systemic effect.

Due to emergency situation that we were part of, healthcare systems around the world were put under tremendous pressure. The focus was on life threatening symptoms. When the pandemic slowed down,

more and more patients reported symptoms atypical for the infection, such as ear, nose, and throat symptoms. It was becoming clear that with severe acute respiratory syndrome coronavirus 2 a much wider variety of symptoms became real, not just respiratory.

It is shown that auditory, vestibular and sensory related symptoms are a part of coronavirus disease 2019 clinical presentation. The fact is, coronavirus disease 2019 affects not only the respiratory tract, and it may do it slowly, without raising too much attention during the infection or even after. Also, it can leave behind subclinical damage that patients may be unaware of, but that can make them more susceptible to damage later on in life, such as differences in tone audiometry and transient evoked otoacoustic emission in asymptomatic patients and healthy individuals.

This paper shows that otorhinolaryngology manifestations are part of the coronavirus disease 2019, even though not all questions are answered. The aim of this paper was to show discrepancies in knowledge and literature regarding this subject and raise interest for future discoveries.

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ASSESSMENT OF THE NURSING WORKLOAD IN INTENSIVE CARE UNITS FOLLOWING CARDIAC SURGERY USING THE NURSING ACTIVITIES SCORE IN RELATION TO REGULATIONS IN SERBIA

PROCENA OPTEREĆENJA MEDICINSKIH SESTARA NA ODELJENJU INTENZIVNE NEGE POSLE KARDIOHIRURGIJE KORIŠĆENJEM SKORA SESTRINSKIH AKTIVNOSTI U ODNOSU NA PROPISE U SRBIJI

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Summary

Introduction. The Nursing Activities Score is used to quantify, assess, and identify the workload of nurses in intensive care units, and is widely considered a very effective, simple, standardized measurement scale, sufficiently detailed to be implemented in everyday hospital practice. The aim of the study is to evaluate the workload of nurses using the results of the Nursing Activities Score and compare the nurse-to-patient ratio with regulations in our country. **Material and Methods.** The study included 131 patients who underwent cardiac surgery in the period from November 1 to November 30, 2021. Surgical data and corresponding Nursing Activities Score values were obtained from hospital information system. In the intensive care unit, nursing activities were monitored and scored daily. **Results.** Using the Nursing Activities Score, 344 measurements were recorded in the study sample. The mean Nursing Activity Score was 88.27 ± 3.63, and the highest was 102.97 ± 1.89 on the first postoperative day. The nurse-to-patient ratio was 1.03:1 on the first postoperative day, and about 0.8:1 on the second and third postoperative days. There was a statistically significant difference in the mean Nursing Activities Score between postoperative days ($p < 0.0005$) between the first and second day (102.96 vs. 79.46) and the first and third day (103.46 vs. 83.58). **Conclusion.** On the first postoperative day, the optimal number of nurses according to the Nursing Activities Score is higher compared to the State Regulations, while for the rest of the days in the intensive care unit the nurse-to-patient ratio corresponds to the regulations.

Key words: Workload; Intensive Care Units; Cardiac Surgical Procedures; Nursing Care; Nursing Staff, Hospital; Government Regulation

Sažetak

Uvod. Skor sestrinskih aktivnosti koristi se za kvantifikaciju, procenu i identifikaciju radnog opterećenja medicinskih sestara u odeljenjima intenzivne nege i uopšte smatra se veoma efikasnom, jednostavnom, standardizovanom skalom, dovoljno detaljnom za primenu u svakodnevnoj bolničkoj praksi. Cilj studije je da proceni opterećenje medicinskih sestara korišćenjem rezultata ovog sistema i uporedi odnos medicinska sestra/pacijent sa regulativom u našoj zemlju. **Materijal i metode.** Istraživanje je sprovedeno među 131 pacijentom, podvrgnutom kardiohirurškoj operaciji, u perodu od 1. do 30. novembra 2021. godine. Hirurški podaci i odgovarajuće vrednosti Skora sestrinskih aktivnosti dobijeni su iz bolničkog informacionog sistema. Na odeljenju za intenzivnu negu, pacijenti su svakodnevno ocenjivani korišćenjem ovog sistema i praćene su sestrinske aktivnosti. **Rezultati.** Korišćenjem Skora sestrinskih aktivnosti zabeležena su 344 merenja u uzorku studije. Srednja vrednost Skora bila je 88,27 ± 3,63, a najviša 102,97 ± 1,89 za prvi postoperativni dan. Prema Skoru, odnos sestra-pacijent za prvi postoperativni dan bio je 1,03 : 1, a za drugi i treći dan 0,8 : 1. Postojala je statistički značajna razlika u srednjim vrednostima Skora između postoperativnih dana ($p < 0,0005$), između prvog i drugog dana (102,96 vs 79,46) i prvog i trećeg (103,46 vs 83,58). **Zaključak.** Za prvi postoperativni dan, potreban broj medicinskih sestara, koji je izračunao Skor sestrinskih aktivnosti, veći je u poređenju sa državnom regulativom; za ostale dane boravka u intenzivnoj nezi, odnos sestre-pacijent odgovara definisanim propisima.

Ključne reči: opterećenje poslom; jedinice intenzivne nege; kardiohirurške procedure; sestrinska nega; bolničko osoblje; državna regulativa

Introduction

Cardiac surgery procedures stand out as some of the most intricate and complex surgical interventions. Patients who have undergone cardiac surgery require specialized care to provide successful postoperative recovery. The introduction of new procedures in everyday clinical practice and on-going advances in surgical treatment and in intensive care units (ICUs) require continuing education of all members of healthcare teams participating in the treatment of these patients [1, 2].

Contemporary cardiac surgery intensive care units (CSICUs) demand enhanced, complete and continuous cardiovascular invasive hemodynamic monitoring.

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Abbreviations
ICU – intensive care unit
CSICUs – cardiac surgery intensive care units
LOS – length of stay
NAS – Nursing Activity Score
POD – postoperative day

Adequate planning, organization, evaluation and analysis of nurse workload are indispensable, not only for determining the optimal number of nurses but also for providing quality health care [3, 4].

Recent results indicate that a reduced number of nurses decrease the quality of health care, which in turn increases the risk of nosocomial infections and decubitus, postoperative complications, mortality, and length of stay (LOS) [5–7]. A constant increase in the number and complexity of nursing activities in ICUs has lead to a significant increase in the nursing workload, as well as establishment of a wide range of methods for its measurement [2, 8].

Various workload scoring systems have been developed to determine the extent of nursing activities in ICUs and to assess the required number of nurses and their workload [9, 10]. Clinical studies showed that nurses spend a higher percentage of time on some activities that were not included in previous scoring systems and the Nursing Activities Score (NAS) was developed in 2003 as a new scale [11]. The score is based on the time consumed by nurse activities at the patient level, regardless of the severity of the health condition of the patient being cared for. It is one of the instruments used to measure the workload of nurses and is considered a very efficient, simple, standardized measurement scale, applicable in everyday hospital practice [12–14].

The study aims to:

Assess the nursing workload in intensive care units after cardiac surgery by using NAS;

Determine the optimal number of nurses per patient in an ICU using NAS and compare it with the state nurse staffing regulations.

Material and Methods

This prospective observational study was conducted in the regional tertiary-care university hospital (Cardiovascular Surgery of the Institute of Cardiovascular Diseases of Vojvodina, Serbia) over a period of a month, between November 1 and 30, 2021 at the Cardiac Surgery Clinic. Data were collected prospectively from patients who underwent coronary or valvular surgery and combined coronary and valvular procedures. Patients with congenital heart defects, aortic dissections, and heart tumors were excluded from the study.

The sample included 131 patients who stayed in the ICU more than six hours. The grading was done daily, in the morning hours before leaving for the department. The data were taken from the hospital information system and analyzed in relation to the type of surgery, age, LOS in the ICU, and the activities of nurses in the ICU after cardiac surgery. During their stay in the ICU, the patients were scored by nurses using the NAS system, which is routinely performed in our ICU, as proposed by Miranda DR. et al. [11]. The NAS system has 23 items ranging from 1 to 177. The NAS above 100 indicates that the needed care can only be provided by more than one nurse. According to the NAS system, the optimal activity of nurses during a 24-hour period per patient equals 100 [15], which equals a nurse-to-patient ratio of 1:1 [15–17].

Statistical data processing was carried out using IBM SPSS 17 software and data analysis was performed using descriptive and inferential statistical methodologies. Numerical features are presented in means (arithmetic mean, median) and in measures of variability (range of values, standard deviation (SD)), and attribute features according to frequency and percentage. Student's t-test and Mann-Whitney test were used to compare differences between the mean values of numerical variables. The level of statistical significance of $p < 0.05$ was considered statistically significant. The results are presented in tabular form.

Results

Out of a total of 131 patients, coronary surgery was performed in 38.2% (50/131), 40.5% of patients underwent valvular surgery (53/131), and 21.4% of patients had combined procedures (28/131). There were less female patients, accounting for 35.9% (47/131) compared to 64.1% (84/131) of male patients.

Table 1. Mean length of stay, length of stay in intensive care unit, age and Nursing Activity Score
Tabela 1. *Srednje vrednosti trajanja hospitalizacije, dužine boravka u jedinici intenzivne nege, godine života i Skor sestrinskih aktivnosti*

	Number of patients *Broj pacijenata*	Minimum *Minimalno*	Maximum *Maksimalno*	Mean/*Srednja vrednost*	SD *SD*
Length of stay (in days) *Dužina ležanja u bolnici (po danima)*	131	7.00	42.00	12.32	6.03
Length of stay in intensive care unit (in days) *Dužina boravka u intenzivnoj jedinici (po danima)*	131	0.68	24.92	1.77	0.45
Age (years)/*Godine života*	131	32	82	66.10	9.13
Cumulative Nursing Activity Score *Zbirni Skor sestrinskih aktivnosti*	131	154.60	893.85	231.90	130.48
Nursing Activity Score/24 h *Skor sestrinskih aktivnosti/24 h*	131	77.30	110.70	88.27	3.63

Legend: SD - standard deviation/*Legenda: SD - standarna devijacija*

Table 2. Comparison of pairs of mean Nursing Activity Scores (NAS) in relation to the postoperative day
Tabela 2. *Poređenje parova srednjih vrednosti Skora sestrinskih aktivnosti (SSA) u odnosu na postoperativni dan*

	NAS/SSA	Number of patients/*Broj pacijenata*	Mean/*Srednja vrednost*	SD/*SD*	p/*p*
Pair 1/*Par 1*	POD1	131	102.96	1.89	< 0.0005
	POD2	131	76.46	5.96	
Pair 2/*Par2*	POD1	35	103.46	1.58	< 0.0005
	POD3	35	83.58	13.25	
Pair 3/*Par 3*	POD2	35	83.07	7.76	0.788
	POD3	35	83.58	13.25	

Legend: SD - standard deviation; POD - postoperative day/*Legenda: SD - standarna devijacija; POD – postoperativni dan*

Table 3. Mean Nursing Activity Scores in the first three postoperative days and nurse-to-patient ratio
Tabela 3. *Srednje vrednosti Skora sestrinskih aktivnosti za tri postoperativna dana, odnos medicinska sestra/pacijent*

Postoperative day *Postoperativni dan*	Number of patients/*Broj pacijenata*	Nursing Activity Score/*Skor sestrinskih aktivnosti*				Nurse-to-patient ratio *Odnos sestra-pacijent*
		Minimum *Minimalno*	Maximum *Maksimalno*	Mean/*Srednja vrednost*	SD *SD*	
POD1	131	94.10	111.30	102.97	1.89	1.03:1
POD2	131	60.50	104.70	76.46	5.96	0.76:1
POD3	35	68.70	138.80	83.58	13.25	0.84:1

Legend: SD - standard deviation; POD - postoperative day/*Legenda: SD – standarna devijacija, POD – postoperativni dan*

The total number of performed NAS measurements for all patients during their LOS in the ICU was 344. The average age was 66.10 ± 9.13 years. The mean NAS was 88.27 ± 3.63, and the mean cumulative value was 231.9 ± 130.48. Mean LOS, age and NAS values are shown in **Table 1.**

On the second and third postoperative day (POD), 35 patients (26.7%) remained in the ICU. The mean NAS on the POD1 was 102.96 ± 1.89, on POD2 it was 76.46 ± 5.96, and on the POD3 it was 83.58 ± 13.25. There was a statistically significant difference between the average NAS on the first and the second day, as well as between the first and the third day ($p < 0.0005$) **(Table 2)**.

Table 3 shows the required number of nurses, i.e. the optimal number of nurses, based on the mean NAS on the first three PODs (nurse-to-patient ratio). During the POD1 each patient needed a nurse for 24 hours (1.03:1); on the following days, the number of required nurses was reduced to 1/5 (0.76:1; 0.84:1).

Discussion

Assessment of the nursing workload and patient needs is a prerequisite for adequate stuffing and optimal allocation of nurses in an ICU since it provides quality service [3, 18, 19]. This is very important for hospital management and planning appropriate number of nurses [7–9].

In several studies, the NAS model was used to measure and analyze the nursing workload in cardiac surgery ICUs. A study [20] undertaken in a tertiary University CSICU, shows that the mean daily NAS was 74.6 points, and the mean value on the POD1 was 96.76. The results of Portuguese researchers [21] showed that the workload of nurses per patient on the POD1 in the ICU was 80.52 ± 10.89, i.e. it was higher compared to the later stay by 19.36 ± 2.61. In our study, the mean daily NAS on the POD1 gave a similar result of 102.94 ± 4.01.

The mean NAS in our study decreased during the ICU course by PODs (POD1 - 102.97, POD3 - 83.58), which suggests that patients needed the most intensive supervision and care following their arrival from the operating room, or on the POD1, which is in agreement with the results of other authors [22].

Similar mean NAS values were also obtained in other studies. In a highly specialized clinic for cardiac surgery, the mean value of NAS was found to be 73.7 [17].

Researchers in Belgium carried out two studies to assess the nursing workload and validate the NAS system in their ICUs [19, 23]. The reason for introducing NAS scoring system was to provide a better quality of healthcare and to assess the nurse-to-patient ratio. The mean NAS values in these studies were 69%, 76% [23], and 68.6% [19]. In addition, they validated the reliability of the NAS in their hospitals and found that it is a valid instrument which allows a reliable assessment of the nursing workload.

The authors [13] conducted a systematic analysis of a large number of articles and found that NAS ranged from a minimum 36.1 in Greece, to maximum 109.3 in Iranian patients, while in Norwegian studies NAS was 96.24 ± 22.35, 88.4 ± 16.2 and 88.7 ± 24.5. In our study, the mean NAS in our population was 88.27 ± 3.63 and it is most similar to the mean NAS obtained in Norway [24].

During the observation period of our study, on the POD1 the mean NAS was 102.97, which implies that the nurse-to-patient ratio was 1.03:1. On the POD2 and POD3, the NAS ranged from 76 - 84 points (on the POD2 it was 76.46, on the POD3 it was higher - 83.58).

Our results show that the number of nurses in relation to the needs of patients according to the NAS system after the POD1 was 20% lower, giving a ratio of about 0.8:1 (0.76:1 on the POD2, 0.84:1 on the POD3). This is understandable, since patients need the most intensive care and supervision immediately upon arrival from the operating room, i.e. on POD1. The results of a Belgian study using the NAS system showed that the optimal nurse-to-patient ratio was 1:1.5 in various ICUs [19].

Healthcare legislation in our country stipulates that the nurse-to-patient ratio in ICUs should be 0.8:1 [25]. In our study, we found that the required number of nurses per patient in CSICU on POD1 is higher than the number of nurses foreseen by the relevant legislation.

According to Belgian legislation, the nurse-to-patient ratio should be 1:3, while the results of a study conducted in several Belgian hospitals showed that the optimal number of nurses should be twice as high [19]. In Norway, a standard nurse-to-patient ratio has been set at 1.3:1 [24].

The assessment of the reliability of the NAS system shows that some of its practical applications may have certain shortcomings, and attempts have been made in some countries to improve this scoring system [16, 26, 27]. Although variations in the results have been observed, the NAS system has been translated into many languages and has been used in many countries to assess the workload of nurses which is an essential component for planning the required number of nurses and their schedules according to individual patient needs [13, 14, 23].

As each ICU has its own characteristics, it is very difficult to provide recommendations for the optimal number of nurses per patient [9, 10, 27, 28]. An analysis of a database containing several similar studies concluded that nurse-to-patient ratios that were calculated using the NAS system were higher than the nurse-to-patient ratios stipulated in the legislation of corresponding countries [12].

Conclusion

The use of the Nursing Activities Score to evaluate nursing workload in intensive care units facilitates an objective assessment of the optimal nurse-to-patient ratio. As per the Nursing Activities Score system, the most significant surge in nursing workload occurs on the initial postoperative day, where the nurse-to-patient ratio slightly surpasses 1:1. This ratio gradually declines during the postoperative recovery period, reaching approximately 0.8:1 on the second and third postoperative days. Remarkably, on the first postoperative day, the number of nurses required as indicated by Nursing Activities Score surpasses the stipulations outlined in our country's regulations.

To validate our findings, further research in the region could provide valuable insights and recommendations regarding potential adjustments to prevailing regulations pertaining to nursing staff prerequisites in both cardiac surgery intensive care and other intensive care units.

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LIPID RESIDUAL CARDIOVASCULAR RISK PARAMETERS IN PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA – IMPORTANCE OF TRIGLYCERIDE TO HIGH-DENSITY LIPOPROTEIN RATIO

LIPIDNI PARAMETRI REZIDUALNOG KARDIOVASKULARNOG RIZIKA KOD PACIJENATA SA FAMILIJARNOM HIPERHOLESTEROLEMIJOM − VAŽNOST ODNOSA TRIGLICERIDA I HOLESTEROLA VELIKE MASE

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Summary

Introduction. Familial hipercholesterolemia is monogenetic disorder associated with early onset of cardiovascular disease. The measurement of low density cholesterol is the primary therapeutic goal in familial hypercholesterolemia patients, but despite the lipid-lowering therapy cardiovascular disease still occurs. It became clear that it's necessary to consider residual cardiovascular risk. The aim of study was to evaluate residual cardiovascular risk in familial hipercholesterolemia. **Material and Methods.** In this cross-sectional study we included 291 familial hipercholesterolemia patients with and without previously diagnosed diabetes. Based on value of the Dutch Lipid Clinical Network score criteria, familial hipercholesterolemia patients without diabetes was further divided into: possible (3-5 points), probable (6-8 points), and definite (>8 points) familial hypercholesterolemia. Triglyceride to high density cholesterol ratio, non-HDL-cholesterol and remnant cholesterol were used as parameters of lipid residual cardiovascular risk. **Results.** We found statistically significant differences in total cholesterol, low and high density cholesterol, triglycerides and apolipoprotein B between the groups ($p>0.05$). The definite and probable group had higher non-HDL-cholesterol values than possible and familial hypercholesterolemia with diabetes ($p<0.01$) groups. Familial hypercholesterolemia with diabetes group had higher values of triglyceride to high density cholesterol ratio and remnant cholesterol than definite and probable group ($p<0.01$). Regression analysis showed that triglyceride to high density cholesterol ratio was independent predictor of appearance of coronary artery disease in addition to elevated low density cholesterol and non-HDL-cholesterol ($p<0.01$). **Conclusion.** Triglyceride to high density cholesterol ratio is the most important parameter of the lipid residual cardiovacular risk that strongly linked with cardiovascular disease in familal hypercholesterolemia patients, especially with associated diabetes.

Key words: Heart Disease Risk Factors; Hyperlipoproteinemia Type II; Diabetes Mellitus; Cardiovascular Diseases; Risk Factors; Triglycerides; Cholesterol, HDL

Sažetak

Uvod. Familijarna hiperholesterolemija je nasledna bolest povezana sa pojavom prematurne kardiovaskularne bolesti. Primarni terapijski cilj je nivo holesterola male molekulske mase, ali se uprkos primeni hipolipemijske terapije često razvija kardiovaskularna bolest. Stoga, postalo je neophodno uzeti u obzir rezidualni kardiovaskularni rizik. Cilj ove studije bio je evaluacija rezidualnog kardiovaskularnog rizika u pacijenata sa familijarnom hiperholesterolemijom. **Materijal i metode.** U ovu presečnu studiju uključili smo 291 pacijenta sa familijarnom hiperholesterolemijom sa i bez prethodno dijagnostikovanog dijabetesa. Na osnovu Dutch Lipid Network Clinical skora za postavljanje kliničke dijagnoze familijarne hiperholesterolemije, grupu pacijenata sa familijarnom hiperholesterolemijom bez dijabetesa smo podelili na: Moguća (3-5 poena), Verovatna (6-8 poena) i Sigurna (>8 poena) familijarna hiperholesterolemija. Odnos triglicerida i holesterola velike molekulske mase, non-HDL-holesterola i holesterolskih ostaka su korišćeni kao lipidni parametri rezidualnog kardiovaskularnog rizika. **Rezultati.** Našli smo statistički značajne razlike u ukupnom holesterolu, holesterolu velike i male molekulske mase, trigliceridima i apolipoproteinu B između grupa ($p>0,05$). Grupe definitivna i verovatna imale su veće vrednosti non-HDL-holesterola od moguće i familijarne hiperholesterolemije sa udruženim dijabetesom ($p<0,01$). Grupa familijarne hiperholesterolemije sa dijabetesom imala je veće vrednosti odnosa triglicerida i holesterola velike molekulske mase i holestrolskih ostaka od Verovatne i Definitivne grupe ($p<0,01$). Regresiona analiza je pokazala da je odnos odnos triglicerida i holesterola velike molekulske mase bio nezavisan prediktor pojave koronarne arterijske bolesti pored povišenih holesterola male molekulske mase i non-HDL-holesterola ($p<0,01$). **Zaključak.** Odnos triglicerida i holesterola velike molekulske mase je najvažniji parametar lipidnog rezidualnog kardiovaskularnog rizika snažno povezan sa nastankom koronarne arterijske bolesti kod pacijenata sa familijarnom hiperholesterolemijom, posebno u slučajevima pridruženog dijabetesa.

Ključne reči: faktori rizika srčanih oboljenja; familijarna hiperholesterolemija; dijabetes melitus; kardiovaskularna oboljenja; faktori rizika; trigliceridi; HDL holesterol

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Abbreviations

FH	– familial hypercholesterolemia
Ch	– total cholesterol
LDL-Ch	– low-density lipoprotein cholesterol
LDLR	– low-density cholesterol lipoprotein receptor
Apo	– apolipoprotein
PCKS9	– proprotein convertase subtilisin/kexin type 9
LDLRAP	– low-density lipoprotein receptor adapter protein
DLCN	– Dutch Lipid Clinic Network
ASCVD	– atherosclerosis cardiovascular disease
CVD	– cardiovascular disease
CV	– cardiovascular
Tg/HDL	– triglyceride to high-density lipoprotein
CAD	– coronary artery disease
SE	– standard error
ANOVA	– analysis of variance
BMI	– body mass index
MetS	– metabolic syndrome
Non-HDL-Ch	– non-high-density lipoprotein cholesterol
DM	– diabetes mellitus

Introduction

Familial hypercholesterolemia (FH) is one of the most common monogenic disorders with a prevalence of 1:250 - 1:500. It is often an underdiagnosed, underestimated and untreated disease that leads to severe elevation of the serum concentrations of total cholesterol (Ch) and low density lipoprotein cholesterol (LDL-Ch). The FH is caused by three main known gene mutations such as defects in LDL receptor (LDLR) gene, less often a variant in the gene for its ligand, apolipoprotein (Apo) B, and rarely by mutation in proprotein convertase subtilisin/kexin type 9 (PCSK9) gene [1–4]. Also, the phenotypic expression with a recessive inheritance may be caused by variants in the LDL receptor adapter protein (LDLRAP) gene [5, 6].

Genetic testing is the gold standard for diagnosing FH; however, it is not always available. The European Association for Atherosclerosis recommends the Dutch Lipid Clinic Network (DLCN) criteria for clinical diagnosis of FH. The DLCN score is useful for clinical evaluation and detection of FH, but it can sometimes be compromised by the lack of information needed to quantify it. As an alternative, the criteria of United States Program - Make Early Diagnosis to Prevent Early Deaths and Simon-Broome Diagnostic criteria can be used [5–7].

The LDL-Ch is the primary target for initiating lipid-lowering therapy including statins, ezetimibe, PCSK9 inhibitors, and LDL-apheresis, in order to prevent the increase of morbidity and mortality of atherosclerotic cardiovascular disease (ASCVD) in FH patients [1, 5, 6, 8, 9]. Although LDL-Ch is a measure of CARDIOVASCULAR DISEASE (CVD) risk and lowering LDL-Ch is the primary therapeutic goal, there is evidence that despite the lipid-lowering therapy CVD still occurs, thus it is necessary to pay attention to the residual cardiovascular (CV) risk [8, 9]. The triglyceride to high-density lipoprotein (Tg/HDL) ratio is a useful marker of cardiometabolic risk strongly associated with coronary artery disease (CAD), cardiovascular events, metabolic syndrome (MetS), insulin resistance and development of diabetes mellitus (DM) [10–14].

Non-HDL-Ch value includes all the atherogenic lipoprotein particles and according to the results of primary and secondary prevention studies, non-HDL-Ch is a predictor of the lipid residual CV risk [8, 15–18].

Remnant-Ch is also proposed as a marker of residual CVD risk. It is considered that there is an association between elevated level of remnant-like particles and an increased risk for myocardial infarction [8, 16–18].

The DM is a metabolic disorder which is closely related with increased risk for CVD complications [19]. Previous studies [20, 21] suggested that patients with both hereditary dyslipidemia and DM are at significantly higher risk for unwanted outcomes of ASCVD than patients who only suffer from DM.

Considering all the above facts, the aim of the study was to evaluate the lipid residual CV risk parameters in FH patients not receiving lipid lowering therapy in order to identify patients at increased residual risk of CVD in whom treatment strategy can be adapted.

Material and Methods

The subjects included in this cross-sectional study were selected from medical records of 291 consecutive patients with FH+ diagnosed by DLCN score. Of these subjects, we selected those without type 2 DM (FH + DM-) and subject with diagnosed type 2 DM (FH + DM+). Based on the value of the DLCN score, the FH + DM- group was further divided into three subgroups: possible FH + DM- (3 - 5 points), probable FH + DM- (6 - 8 points), and definitive FH + DM- (> 8 points). The exclusion criteria for participation were: a) individuals < 18 years; b) incomplete medical documentation; c) previous use of statins in therapy. The data collected refer to the first examination, i.e., before the start of lipid therapy.

Dutch Lipid Clinic Network score criteria

The DLCN score is a set of criteria including the following data: family history of early CVD (for women > 60 years, for men > 55 years), the presence of corneal arcus and xanthomas, and individuals younger than 18 years with serum LDL-Ch values above the 95th percentile for age and sex; personal history of prior coronary, cerebral, or peripheral vascular disease; blood LDL-Ch levels before initiation of therapy.

Lipid parameters for the residual cardiovascular risk

We used Tg/HDL ratio, non-HDL-Ch and remnant-Ch as parameters of the residual cardiovascular risk.

The Tg/HDL ratio was calculated as the quotient of serum triglyceride and HDL-Ch levels. The value of 1.0 for men and 1.3 for women was indicated as the cut-off value for identifying patients at high CVD risk. Values higher than these indicated presence of an atherogenic lipid profile, which is associated with hypertension, hypercholesterolemia and hypertriglyceridemia which are significant risks for development of CVD [14].

Non-HDL-Ch was calculated from a lipid profile (non-HDL-Ch = Ch minus HDL-Ch. Non-HDL-Ch can

be calculated in a non-fasting patient, unlike LDL-Ch measurement, which requires fasting. The optimal non-HDL cholesterol is less than 3.3 mmol/L so a higher value indicates a higher risk for heart disease [15].

Remnant-Ch was calculated from a lipid profile (remnant-Ch = Ch minus LDL-Ch minus HDL-Ch. Elevated remnant cholesterol concentrations were defined as ≥1.0 mmol/L [16, 17].

Laboratory tests and anthropometric measurements were performed at the Clinic of Endocrinology, Diabetes and Metabolic Diseases of the University Clinical Centre of Serbia. Blood samples were collected after 12 - 14 hours of overnight fasting. Lipid status was assessed by determining Ch, HDL-Ch, and triglycerides using an enzymatic colorimetric assay for quantitative determination on Olympus analyzer. The LDL-Ch was calculated by using the Friedewald equation. Apolipoproteins (Apo) B and A1 were determined by the immunoturbidimetry method.

The data were previously tested for normality of distribution by Kolmogorov-Smirnov test and presented as a mean ± standard error (SE) or median. The differences between FH+ DM- and FH + DM+ groups were tested by using a one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, while the Pearson chi-square test was used for categorical variables. In order to analyze the independent determinants of CVD, binary logistic regression analysis was used, with CVD as dependent variable. The statistical analyses were performed using SPSS software, version 20.0 and differences were considered statistically significant if $p < 0.05$.

Results

The characteristics of subjects included in the study are summarized in **Table 1**. We found that there were statistically significant differences between groups in gender, age, body mass index (BMI) and incidence of CVD ($p < 0.05$). In all groups there were more female patients ($p < 0.01$). In our study, FH + DM+ patients were older than definite FH + DM- patients ($p = 0.01$) and probably FH + DM- patients ($p < 0.001$). Also, possible FH + DM- patients were older than probably FH + DM- patients ($p = 0.03$). We found that FH + DM+ group had a higher BMI than definitive FH + DM-, probable FH + DM- and possible FH + DM- ($p < 0.05$) groups. The possible FH + DM- and FH + DM+ patients had higher prevalence of CVD than definitive FH + DM- and probable FH + DM- patients ($p < 0.001$).

Lipid parameters in FH patients with and without diabetes are summarized in **Table 2**. We observed that there were statistically significant differences in Ch, LDL-Ch, HDL-Ch, triglycerides and Apo B between groups ($p > 0.05$). The definitive FH + DM- group had higher Ch values than possible FH + DM- and FH + DM+ ($p < 0.01$) groups. The probable FH + DM- had higher Ch values than possible FH + DM- and FH + DM+ groups ($p = 0.03$). The definitive FH + DM- group had higher LDL-Ch values than possible FH + DM- and FH + DM+ ($p < 0.01$) groups. The probable FH + DM- group had higher LDL-Ch values than possible FH + DM- and FH + DM+ groups ($p < 0.01$). The FH + DM+ group had higher triglycerides than definitive FH + DM- ($p < 0.01$) and probable FH + DM- groups ($p = 0.02$). The definitive FH + DM- group had higher Apo B values than possible FH + DM- group ($p < 0.01$). The probable FH + DM- group had higher Apo B values than possible FH + DM- group ($p < 0.01$).

Parameters of residual cardiovascular risk of lipid origin in FH patients with and without diabetes are summarized in **Table 3**. We found statistically significant differences in values of parameters of residual CVD risk of lipid origin between the groups ($p > 0.05$). The group with definitive FH + DM- had higher non-HDL values than possible FH + DM- and FH + DM+ groups ($p < 0.01$). The probable FH + DM-

Table 1. Patient characteristics
Tabela 1. *Karakteristike pacijenata*

	FH + DM-			FH + DM+	p / *p*
	Definitive/*Sigurna* Group I/*Grupa I*	Probable/*Verovatna* Group II/*Grupa II*	Possible/*Moguća* Group III/*Grupa III*	Group IV *Grupa IV*	
n	48	69	122	52	
Gender/*Pol* (m/f/*m/ž*)	11/37	18/51	46/76	19/33	*< 0.01
Age/*Starost*[a]	53.8 ± 14.9	52.9 ± 16.7	61.4 ± 14.3	62.0 ± 12.8	*< 0.001
BMI (kg/m^2)/*ITM*[b] *(kg/m^2)*	25.3 (18.9 - 34.2)	24.8 (19.4 - 35.8)	24.9 (18.3 - 38.5)	28.3 (23.0 - 39.4)	**< 0.05
CAD (%)/*KBS (%)*	9.8%	9.8%	41.2%	39.2%	¶< 0.001

[a]Data are expressed as a mean ± SE or as a [b]median/*[a]Podaci su izraženi kao srednja vrednost ± standardna greška ili kao [b]medijana*; BMI/ITM - Body mass index/*Indeks telesne mase*; CAD - Coronary artery disease/*KBS - koronarna bolest srca*

DLCNS – Dutch Lipid Clinic Network score/*Skor*; FH + DM- – Familial hypercholesterolemia patients without diabetes/*Pacijenti sa familijarnom hiperholesterolemijom bez dijabetesa*; FH + DM+ – Familial hypercholesterolemia patients with diabetes/*Pacijenti sa familijarnom hiperholesterolemijom i udruženim dijabetesom*

Group/*Grupa I* – DLCNS > 8; Group II/*Grupa II* – DLCNS 6 – 8; Group III/*Grupa III* – DLCNS 3 – 5; Group IV/*Grupa IV* – DLCNS ≥ 3 with type 2 diabetes mellitus/*sa tipom 2 dijabetesa*

**p – One way ANOVA test for differences between Group I, Group II, Group III and Group IV/*Jednofaktorska analiza varijanse je korišćena kao test za razliku između I, II, III i IV grupe. Bonferonijev naknadni test razlike između grupa*

****p – Kruskal-Wallis test used for differences between Group I, Group II, Group III and Group IV/*Kruskal-Volisov test između I, II, III i IV grupe.*

¶p – Chi-square test between Group I, Group II, Group III and Group IV/*Pirsonov Hi-kvadratni test između grupa*

Table 2. Lipid parameters in FH patients with (FH + DM+) and without diabetes (FH + DM-)
Tabela 2. *Lipidni parametri kod pacijenata sa familijarnom hiperholesterolemijom sa (FH+DM+) i bez dijabetesa (FH+DM-)*

	FH + DM-			FH + DM+	p/*p*
	Definitive/*Sigurna* Group I/*Grupa I*	Probable/*Verovatna* Group II/*Grupa II*	Possible/*Moguća* Group III/*Grupa III*	Group IV *Grupa IV*	
Total Ch/*Ukupni-h (mmol/L)*[a]	8.48 ± 2.74	7.78 ± 2.03	6.96 ± 1.51	6.79 ± 1.91	< 0.01*
HDL-Ch/h (mmol/L)	1.5 ± 0.48	1.41 ± 1.38	1.5 ± 0.48	1.27 ± 1.36	0.01
LDL-Ch (mmol/L)	4.63 ± 1.35	5.52 ± 1.85	4.63 ± 1.35	4.46 ± 1.75	< 0.01
Triglicerydes/*Trigliceridi (mmol/L)*[b]	1.5 (0.6 - 12.4)	1.6 (0.5 - 7.7)	1.8 (0.5 - 11.4)	2.3 (0.9 - 4.1)	< 0.01**
Apo B (g/L)	1.6 (0.2 - 3.0)	1.6 (0.3 - 2.9)	1.4 (0.5 - 2.4)	1.4 (0. 2.7)	< 0.01
Apo A1 (g/L)	1.5 (0.8 - 2.6)	1.7 (1.1 - 2.9)	1.6 (1.0 - 2.7)	1.6 (1.1 - 2.7)	> 0.05
Lp (a) (g/L)	0.3 (0.1 - 1.2)	0.2 (0.1 - 1.6)	0.2 (0.1 - 1.7)	0.2 (0.1 - 1.5)	> 0.05

[a]Data are expressed as mean ± SE or as [b]median; [a]*Podaci su izraženi kao srednja vrednost ± standardna greška ili kao* [b]*medijana*;
DLCNS – Dutch Lipid Clinic Network score/*Skor*
Group/Grupa I – DLCNS > 8; Group/*Grupa* II – DLCNS 6 – 8; Group/*Grupa* III – DLCNS 3 – 5; Group/*Grupa* IV – DLCNS ≥ 3 with diabetes mellitus type 2/*Sa tipom 2 dijabetesa*
Total Ch/*Ukupni-h* – Total cholesterol/*Ukupni holesterol*; HDL-Ch/h – High density cholesterol/*Holesterol velike molekulske mase*; LDL-Ch/h – Low density cholesterol/*Holesterol male molekulske mase*
***** p - One way ANOVA test between Group I, Group II, Group III and Group IV. *Jednofaktorska analiza varijanse je korišćena kao test za razliku između I, II, III i IV grupe. Bonferonijev naknadni test razlike između grupa*
******p – Kruskal-Wallis test between Group I, Group II, Group III and Group IV/*Kruskal-Volisov test između I, II, III i IV grupe.*

Table 3. Parameters of the residual cardiovascular risk in FH patients with (FH + DM+) and without diabetes (FH + DM-)
Tabela 3. *Parametri rezudualnog kardiovaskularnog rizika kod pacijenata sa familijarnom hiperholesterolemijom sa (FH+DM+) i bez dijabetesa (FH+DM-)*

	FH + DM-			FH + DM+	p/*p*
	Definitive/*Sigurna* Group I/*Grupa I*	Probable/*Verovatna* Group II/*Grupa II*	Possible/*Moguća* Group III/*Grupa III*	Group IV *Grupa IV*	
Non-HDL cholesterol/ *holesterol* (mmol/l)[b]	7.5 (1.8 - 12.4)	6.5 (2.9 - 11.4)	5.7 (2.0 - 9.8)	5.6 (2.5 - 11.8)	*< 0.01
Remnant-cholesterol *Rezidualni holesterol* (mmol/l)	0.7 (0.3 - 8.5)	0.7 (0.2 - 8.1)	0.8 (0.2 - 9.8)	1.1 (0.5 - 2.6)	*< 0.01
Tg/HDL ratio/*odnos*	1.1 (0.4 - 9.1)	1.2 (0.3 - 8.5)	1.5 (0.3 - 12.3)	1.7 (0.8 - 4.2)	*< 0.01

Data are expressed as [b]median;*Podaci su izraženi kao* [b]*medijana*
Group/*Grupa I* – DLCNS > 8; Group/*Grupa* II – DLCNS 6 – 8; Group/*Grupa* III – DLCNS 3 – 5; Group/*Grupa* IV – DLCNS ≥ 3 with diabetes mellitus type 2/*Sa tipom 2 dijabetesa*
*****p – Kruskal-Wallis test between Group I, Group II, Group III and Group IV/*Kruskal-Volisov test između I, II, III i IV grupe*

group had higher non-HDL-Ch values than possible FH + DM- and FH+DM+ groups (p = 0.03). In our study, the FH + DM+ group had higher values of remnant-Ch than definite FH + DM- and probable groups (p < 0.01). We found that the FH + DM+ group had higher Tg/HDL ratio than definitive FH + DM- and probable FH+DM- (p < 0.01) groups.

In order to evaluate the effects of the investigated determinants on the incidence of CVD in FH+ patients with and without type 2 DM, we performed a binary logistic regression analysis with CVD as a dependent variable and found that the Tg/HDL ratio is a strong independent predictor of CVD in the whole sample and in FH + DM+ patients **(Figure 1)**.

Discussion

Despite the significantly elevated LDL-Ch and non-HDL-Ch in all FH patients, the results of our study show that, if the Tg/HDL ratio is elevated there is a significantly higher risk for early development of CAD. Moreover, our results imply that this association is especially pronounced if there is also DM along with FH. Additionally, of the three investigated residual risk parameters, our results indicate that Tg/HDL is the most important parameter of the lipid residual CV risk, suggesting that in addition to routinely measured LDL-Ch levels in FH patients, attention needs to be paid to the residual risk, as well as the timely diagnosis of DM in those patients.

The CAD is the most common heart disease and represents a consequence of a partial or total vessel obstruction of coronary arteries caused by lipid accumulation and atherosclerotic plaque formation below the tunica intima [22]. Although the high cost of multiple markers of lipid panels which are strongly associated with development of CAD and necessity

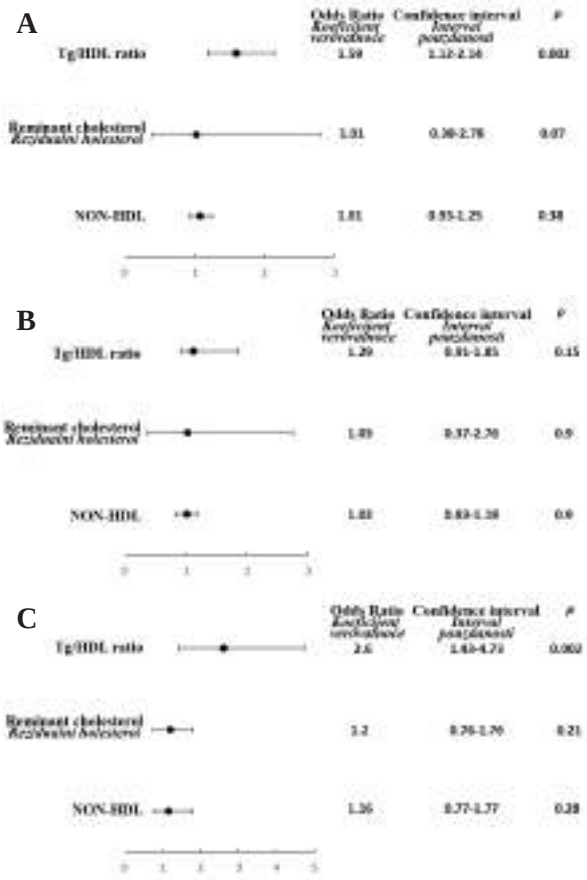

Figure 1. Binary logistic regression analysis of the lipid residual risk determinants in the prediction of CAD in all investigated patients with FH (A), in patient with FH without DM (B) and in patients with FH and DM (C)
Slika 1*. Binarna logistička regresiona analiza determinanti rezidualnog lipidnog kardiovaskularnog rizika za predikciju koronarne bolesti srca kod svih pacijenata (A), kod pacijenata sa familijarnom hiperholesterolemijom bez dijabetes melitusom (B) i kod pacijenata sa familijarnom hiperholesterolemijom bez dijabetes melitusa (C)*

for technical expertise render, researchers have focused to find more affordable atherogenic indices [10].

Previous studies, including our own, show that a high percentage of patients with FH do not reach the recommended LDL-Ch target values, but also that there is a high residual CV risk [3, 9]. Gaziano et al. [13] were the first to point out in a case-control study that Tg/HDL ratio strongly predicts the risk of myocardial infarction, while other studies associate high values of the Tg/HDL with CAD [10–12], degree of coronary atherosclerosis [12] and metabolic syndrome (MetS), so it could be a good marker of increased residual CV risk [10]. In our study, the highest values of Tg/HDL ratio were found in FH patients with associated type 2 DM, and this group also had a high prevalence of CAD. Our results are consistent with the results of numerous studies that have closely linked the high values of Tg/HDL ratio with adverse CV events.

According to our results, patients with FH and type 2 DM had the highest values of triglycerides and the lowest value of HDL-Ch. Our results are consistent with early studies which proved atherogenic lipid profile in diabetic subjects. Insulin deficiency and insulin resistance, two underlying conditions in type 2 DM, lead to dyslipidemia by affecting hepatic lipoprotein production, particularly very low-density lipoprotein and triglyceride-rich lipoproteins. Also, insulin resistance, obesity, MetS and type 2 DM are associated with increased production of serum triglycerides which are associated with low levels of HDL-Ch. Atherogenic dyslipidemia in such conditions is typically characterized by lipid abnormalities such as hypertriglyceridemia, reduced levels of HDL-Ch and increased level of small density LDL particles and Apo B, but often without significant elevation of LDL-Ch [23, 24]. Large-scale epidemiological studies indicated that, regardless of the use of statin therapy, elevated triglycerides levels may be the cause of adverse cardiovascular events. Atherogenic dyslipidemia underlies the residual CV risk that exists despite lowering LDL-Ch to target values [11].

Interestingly, in our results, the possible FH group had higher prevalence of CAD than probable and definite FH groups. Although there were no statistically significant differences in Tg/HDL values, the possible FH group had higher mean value of this ratio than definite and probable FH groups. This result can be explained by the fact that due to unavailable genetic testing for FH, this group may include individuals who do not actually have true hereditary hypercholesterolemia, but MetS. The most common lipid abnormalities associated with MetS are hypertriglyceridemia and low HDL-Ch values as a lipid profile seen in diabetic individuals [25]. The mentioned study [25] showed that Tg/HDL ratio could be an excellent novel marker for effective risk prediction for MetS and CVD.

The results of our study show that the highest remnant-Ch levels were in the group of FH patients with diabetes and it was associated with a high incidence of CAD. Earlier studies [16–18] showed that elevated remnant-Ch level was a significant and independent risk factor for CAD and type 2 DM. Due to small size, remnant-Ch passes easily through the endothelium, then binds to connective matrix in sub-endothelium and promotes chronic low-grade inflammation of smooth muscle cells, thus playing a crucial role in pathological process of atherogenesis [18]. Residual CV risk has been pointed out by various associations, such as the American College of Cardiology, American Heart Association, European Society of Cardiology, and European Atherosclerosis Society. The recommendations of these associations are based on finding a secondary treatment target that may indicate more intensive lipid-lowering therapy. The LDL-Ch remains the primary treatment target, but secondary treatment targets depend on the calculated risk of fatal ASCVD estimated by the score system (very high, high, moderate or low) and also by the associated diabetes [15]. The highest levels of non-HDL-Ch in our study were in the definitive FH group.

The obtained results are the consequence of the highest concentration of LDL-Ch and total-Ch in definitive FH group. Moreover, our study shows that despite the highest levels of LDL-Ch and non HDL-Ch unwanted cardiovascular events were not the most prevalent in this group. A possible reason is that the diagnosis of FH in definitive and probable FH groups was made more often due to the characteristic stigmata (tendon xanthomas, arcus cornealis) than to abnormality in laboratory test results and positive family history (they are not index cases).

Studies [20, 21] also confirmed that FH subjects with diabetes confer a substantial increase in the incidence of adverse CV outcomes than individuals who have only hereditary hypercholesterolemia or only type 2 DM. Due to all of the above, timely diagnosis of these disorders is extremely important, as well as the definition of therapeutic goals with special reference to the residual CV risk [20].

Conclusion

Data from the present study suggest that, regardless of significantly elevated low density lipoprotein and non-high-density lipoprotein cholesterol in all familial hypercholesterolemia patients, if the triglyceride to high-density lipoprotein ratio is elevated, there is a significantly higher risk of coronary artery disease. In addition, our results show that triglyceride to high-density lipoprotein is the most important parameter of the lipid residual cardiovascular risk implying that its measurement is very important in the assessment of overall cardiovascular risk in patients with familial hypercholesterolemia, especially in cases with associated diabetes.

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INTERIM POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY AS A MAJOR PROGNOSTIC FACTOR OF TREATMENT OUTCOME IN NEWLY DIAGNOSED HODGKIN'S LYMPHOMA AND OPTIMIZATION OF THE THERAPEUTIC APPROACH

INTERIM SKEN POZITRONSKE EMISIONE TOMOGRAFIJE – KOMPJUTERIZOVANE TOMOGRA- FIJE KAO GLAVNI PROGNOSTIČKI FAKTOR U PREDIKCIJI ISHODA LEČENJA NOVODIJAGNOSTIKOVANOG HOČKINOVOG LIMFOMA I OPTIMIZACIJI TERAPIJSKOG PRISTUPA

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Summary

Introduction. During the last decade, the interim positron emission tomography/computed tomography has emerged as the most important prognostic factor in patients with newly diagnosed Hodgkin's lymphoma. The aim of this study was to analyze the treatment of patients at the Clinic of Hematology, University Clinical Center of Vojvodina, in order to evaluate the prognostic value of key risk factors, with a particular focus on the role of interim positron emission tomography/computed tomography. **Material and Methods.** The study included 22 of 29 patients with a newly diagnosed classic Hodgkin's lymphoma, i.e. all patients in whom the first therapy response assessment was based on positron emission tomography/computed tomography scan results. Relevant data were collected from medical records. Kaplan-Meier curves and log-rank tests were used for survival analysis. Univariate Cox regression was used to assess the predictive value of each variable for progression-free survival. **Results.** The study included 13 women (59.09%) and 9 men (40.91%), aged 18 to 73 years (median 39.5 years). Univariate analysis was used to analyze the statistical significance of three examined variables: gender, presence of B symptoms, and complete remission on interim positron emission tomography/computed tomography. Multivariate analysis was not performed due to the insufficient number of patients for adequate interpretation of results. **Conclusion.** Interim positron emission tomography/computed tomography is the main prognostic factor in predicting treatment response and disease outcome in patients with newly diagnosed Hodgkin's lymphoma.

Key words: Positron Emission Tomography Computed Tomography; Prognosis; Treatment Outcome; Hodgkin Disease; Lymphoma

Sažetak

Uvod. Tokom poslednje decenije interim sken pozitronske emisione tomografije – kompjuterizovane tomografije izdvojen je kao najznačajniji prognostički faktor kod bolesnika sa novodijagnostikovanim Hočkinovim limfomom. Cilj ovog rada bila je analiza lečenja bolesnika na Klinici za hematologiju Univerzitetskog kliničkog centra Vojvodine, u cilju procene prognostičkog značaja glavnih faktora rizika, sa posebnim osvrtom na ulogu interim skena pozitronske emisione tomografije – kompjuterizovane tomografije. **Materijal i metode.** U istraživanje je uključeno 22 od 29 novodijagnostikovanih bolesnika sa klasičnim Hočkinovim limfomom, odnosno svi bolesnici kod kojih je prva procena terapijskog odgovora sprovedena na osnovu nalaza skena pozitronske emisione tomografije – kompjuterizovane tomografije. Relevantni podaci su prikupljeni iz medicinske dokumentacije. Preživljavanje je analizirano Kaplan-Majerovim krivama, a poređeno log-rank testom. Za procenu prediktivne vrednosti pojedinih varijabli u odnosu na vreme bez progresije bolesti korišćena je univarijantna Koksova regresija. **Rezultati.** Uzorak bolesnika se sastojao od 13 žena (59,09%) i 9 muškaraca (40,91%), starosti od 18 do 73 godine (medijana 39,5 godina). Univarijantnom analizom dobijena je statistička značajnost kod tri ispitivane varijable: pol, prisustvo B-simptoma i prisustvo kompletne remisije na interim skenu pozitronske emisione tomografije – kompjuterizovane tomografije. Multivarijantna analiza nije sprovedena s obzirom na nedovoljan broj bolesnika za adekvatno tumačenje rezultata. **Zaključak.** Interim sken pozitronske emisione tomografije – kompjuterizovane tomografije predstavlja glavni prognostički faktor u predikciji terapijskog odgovora i ishoda bolesti kod bolesnika sa novodijagnostikovanim Hočkinovim limfomom.

Ključne reči: PET/CT; prognoza; ishod lečenja; Hočkinova bolest; limfom

Introduction

Clinical stage of the disease and the International Prognostic Score (IPS) have been considered the most important prognostic factors in predicting the disease outcome at the initial diagnosis of Hodgkin's lymphoma for many years. However, during the last decade, interim positron emission tomog-

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Abbreviations

IPS	– International Prognostic Score
PET/CT	– positron emission tomography/computed tomography
CRP	– C-reactive protein
ABVD	– doxorubicin, bleomycin, vinblastine, dacarbazine
PFS	– progression free survival
OS	– overall survival
FDG	– fluorodeoxyglucose
eBEACOPP	– escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone
SD	– standard deviation

raphy/computed tomography (PET/CT) has been singled out as the most important prognostic factor and the best early predictor of the therapeutic effect. Accordingly, the latest Hodgkin's lymphoma treatment guidelines recommend the therapy selection and modification based on interim PET/CT scan results [1]. Moreover, several authors have recently challenged the prognostic significance of IPS in the era of therapy optimization based on interim PET/CT scan findings.

Against this backdrop, the aim of the present study was to analyze the treatment provided to patients with newly diagnosed Hodgkin's lymphoma at the Clinic of Hematology, University Clinical Center of Vojvodina, in order to evaluate the prognostic significance of key risk factors in this cohort, as well as the correlation between these factors, with a particular focus on the role of interim PET/CT in the selection and modification of therapeutic modality.

Material and Methods

The study included all patients with a newly diagnosed classic Hodgkin's lymphoma who were treated at the Clinic of Hematology, University Clinical Center of Vojvodina, from the beginning of 2016 to the end of 2020, and in whom the first therapy response assessment was based on PET/CT scan results. Patients in whom the first assessment of therapy response was based on CT scan findings were not included in the study.

Hodgkin's lymphoma was diagnosed by pathohistological and immunohistochemical examination of lymph node, bone marrow, and extranodal organ biopsies, in accordance with the current World Health Organization classification [1, 2]. The clinical stage of the disease was determined according to the Ann-Arbor classification [3] and the IPS was calculated [3]. The following data were collected from patients' medical records: age, gender, presence of B symptoms, evidence of bone marrow infiltration, presence of bulky disease, and values of the laboratory parameters before starting the therapy: blood count with differential blood count, C-reactive protein (CRP), fibrinogen, and serum albumin. Bulky disease was defined as the presence of lymph node conglomerate on the initial CT or PET/CT scan greater than 7 cm in the transverse or frontal plane. In all patients, treatment commenced with 2 cycles of doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) and the first revision was performed with PET/CT scan. Progression free survival (PFS) was defined as the time elapsed from diagnosis to disease progression or relapse, or to death from any cause, i.e., to the date of the last contact with a patient in those in whom disease progression did not occur. Overall survival (OS) was defined as the time elapsed from diagnosis to death, i.e., if the patient was still alive, the time period from diagnosis to the last contact with the patient.

Statistical analysis was conducted using the MedCalc Statistical Software version 19.5.1. The D'Agostino-Pearson test was performed to verify the distribution normality of the examined variables. For all normally distributed variables, the data were presented as measures of average values, i.e., as arithmetic mean and standard deviation (SD). The CRP was the only variable that did not conform to the normal distribution; therefore, its values were presented as the median, minimum, and maximum. Limit values for CRP, albumin, and age at diagnosis relative to PFS were determined by receiver operating characteristic curve analysis, whereas Kaplan-Meier curves and log-rank test were used for survival analysis. Univariate Cox regression analysis was conducted to assess the predictive value of each variable for PFS. Hypotheses were accepted or rejected with a risk set at $p < 0.05$ (95% probability).

Results

The research sample included 22 of 29 patients with newly diagnosed Hodgkin's lymphoma who were treated at the Clinic of Hematology, University Clinical Center of Vojvodina, during the study period. The sample included 13 women (59.09%) and 9 men (40.91%), aged 18 to 73 years. The average age of patients was 22.4 years (SD 15.14), and the median age was 39.5 years.

The distribution of patients according to the clinical stage of the disease at diagnosis, as well as the prevalence of examined prognostic factors in relation to the clinical stage of the disease, are shown in **Table 1**. Based on the calculated IPS values, which did not exceed 4 **(Table 2)**, there were no patients in the high-risk group. The limit values of the following parameters were identified by ROC analysis: CRP $\leq$ 7.6 mg/L, albumin > 40 g/L, and $\leq$ 57 years old at initial diagnosis. The patient's follow-up time ranged from 7 to 49 months, average 23.68 months (SD 12.77), and median follow-up 21 months. During that period, disease progression occurred in 6 patients (27.27%), on average after 4.5 months (SD 2.98), median 4 months. The mean PFS was 22.4 ± 15.14 months, while the mean OS was 27.7 ± 12.7 months. Considering that only one patient died during the follow-up period, the censored survival time (time elapsed from diagnosis to the last contact with patient) was used when calculating PFS and OS for the remaining 21 patients. The number of patients who progressed in relation to the analyzed prognostic factors for PFS, as well as the

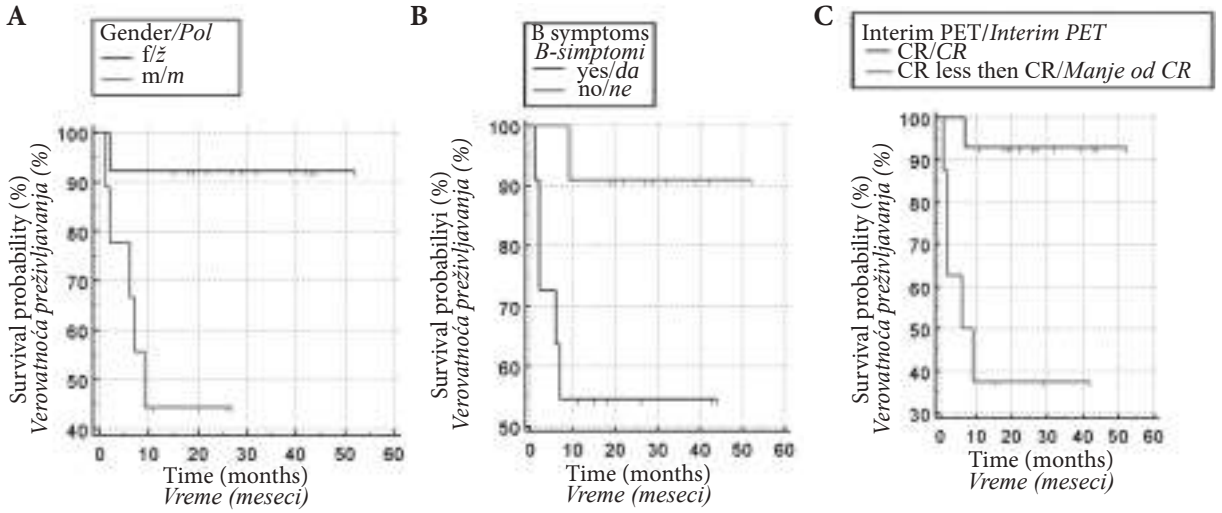
Table 1. Distribution of patients in relation to the clinical stage of the disease at diagnosis and distribution of the prevalence of examined prognostic factors in relation to the clinical stage
Tabela 1. *Raspodela bolesnika u odnosu na klinički stadijum bolesti pri postavljanju dijagnoze i raspodela zastupljenosti ispitivanih prognostičkih faktora u odnosu na klinički stadijum bolesti*

	Total number of patients *Ukupni broj bolesnika*	Stage I *I stadijum*	Stage II *II stadijum*	Stage III *III stadijum*	Stage IV *IV stadijum*
Clinical stage at diagnosis *Klinički stadijum pri postavljanju dijagnoze*	22	0	11 (50%)	8 (36.36%)	3 (13.63%)
B symptoms/*B-simptomi*	11 (50%)	NA	3	5	3
Bulky disease/*Bulky bolest*	7 (31.81%)	NA	1	4	2
EN localization of the disease *EN lokalizacija bolesti*	7 (31.81%)	NA	2	2	3
Bone marrow infiltration *Infiltracija koštane srži*	2 (9.09%)	NA	0	0	2

Legend: NA – Not applicable; EN - Extranodal/*Legenda: NA – Nije primenjivo; EN – Ekstranodalno*

Table 2. International Prognostic Score values
Tabela 2. *Vrednosti Međunarodnog prognostičkog skora (MPS)*

IPS/*MPS*	0	1	2	3	4	5 - 7	
No/*Broj* (%)	3 (13.64%)	6 (27.27%)	7 (31.82%)	1 (4.54%)	5 (22.73%)	0 (0%)	22 (100%)



Graph 1. Kaplan-Meier curves showing survival analysis in regard to gender (A), B symptoms (B) and complete remission (CR) on interim PET/CT scan (C)
Grafikon 1. *Kaplan-Majerove krive koje prikazuju analizu preživljavanja u odnosu na pol (A), B-simptome (B) i kompletnu remisiju (CR) na interim skenu pozitronske emisione tomografije – kompjuterizovane tomografije (C)*

results of the univariate analysis that estimated the predictive value of each prognostic factor, are shown in **Table 3**. Statistical significance was analyzed for three examined variables, namely gender, presence of B symptoms, and evidence of complete remission on interim PET/CT **(Table 3)**. Survival analysis for these three variables is shown on Kaplan-Meier curves **(Graph 1)**. Adequate multivariate analysis requires at least ten events per prognostic factor, while in this study, disease progression occurred in only six patients. Considering the insufficient number of patients, multivariate analysis was not performed.

Discussion

Retrospective analysis of the disease course in patients with newly diagnosed Hodgkin's lymphoma treated at the University Clinical Center of Vojvodina showed that early assessment of therapy response based on interim PET/CT scan results, as ell as individual therapy adjustments in accordance with these results, is important for achieving and maintaining remission, given that the interim PET/CT scan results were identified as the most statistically significant prognostic factor in predicting disease outcome. The prognostic value of interim PET/CT scan for PFS in patients with Hodgkin's lymphoma has also been reported by other authors [4–

Table 3. Patients with disease progression in relation to prognostic factors; predictive value of prognostic factors for progression free survival
Tabela 3. *Bolesnici kod kojih je došlo do progresije bolesti u odnosu na prognostičke faktore, kao i prediktivna vrednost prognostičkih faktora u odnosu na preživljavanje bez progresije bolesti*

Prognostic factor/*Prognostički faktor*						
Gender/*Pol*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Male/*Muški*	5	4	9	22	8.1254 (1.5061 - 43.8356)	0.02
Female/*Ženski*	1	12	13			
B symptoms *B-simptomi*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Yes/*Da*	5	6	11	22	0.1895 (0.03687 - 0.9739)	0.04
No/*Ne*	1	10	11			
CR on interim PET/CT scan *CR na interim PET/CT skenu*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Yes/*Da*	1	13	14	22	14.9818 (2.5039 - 89.6431)	0.003
No/*Ne*	5	3	8			
Age at diagnosis *Starost pri postavljanju dijagnoze*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
> 57 years old/> *57 godina*	0	4	4	22	NA	0.21
≤ 57 years old/≤ *57 godina*	6	12	18			
Albumin level *Vrednost albumina*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
> 40 g/L	5	8	13	18	0.4789 (0.08461 - 2.7111)	0.41
≤ 40 g/L	1	4	5			
CRP level *Vrednost CRP-a*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
≤ 7.6 mg/L	1	7	8	21	2.7232 (0.5247 - 14.1347)	0.23
> 7.6 mg/L	5	8	13			
EN localization of the disease *EN lokalizacija bolesti*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Yes/*Da*	3	4	7	22	2.7044 (0.4616 - 15.8454)	0.27
No/*Ne*	3	12	15			
Bulky disease *Bulky bolest*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Yes/*Da*	2	5	7	19	1.3408 (0.2048 - 8.7782)	0.76
No/*Ne*	3	9	12			
Bone marrow infiltration *Infiltracija koštane srži*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Yes/*Da*	1	1	2	18	3.9877 (0.1813 - 87.7098)	0.38
No/*Ne*	5	11	16			
Advanced stage of the disease *Uznapredovali stadijum bolesti*	DP/*DP*		Total number of patients *Ukupan broj bolesnika*		HR (95% CI)	p/*p*
	Yes/*Da*	No/*Ne*				
Yes/*Da*	6	10	16	22	NA	0.09
No/*Ne*	0	6	6			

Legend: DP - Disease progression; PFS - Progression free survival; HR - Hazard ratio; CI - Confidence interval; p - Probability value ($p < 0.05$ – result is statistically significant); CR - Complete remission; EN - Extranodal; NA - Not applicable
Legenda: DP – progresija bolesti; PFS – preživljavanje bez progresije bolesti; HR – Indeks rizika; CI – interval poverenja; p – vrednost verovatnoće ($p < 0.05$ – postoji statistička značajnost); CR – kompletna remisija; EN – Ekstranodalno; NA – Nije primenjivo

8]. Based on a recent meta-analysis of studies assessing the predictive value of interim PET/CT scan on PFS after two cycles of chemotherapy, Terasawa et al. noted that the three-year PFS in patients with negative interim PET/CT scan results was 90 - 95%, declining to 12 - 30% in patients with positive interim PET/CT scans [9].

High PET/CT predictive value can probably be explained by high sensitivity of Hodgkin's lymphoma to chemotherapy. Therefore, in patients with a good therapy response, a decrease in metabolic activity would manifest on the PET/CT scan immediately after initiation of the chemotherapy protocol [10]. A good fluorodeoxyglucose (FDG) avidity of Hodgkin's lymphoma is attributed to the pathohistological substrate consisting of only 1% of malignant cells, while the majority of tumors comprise a surrounding inflammatory infiltrate with high metabolic activity that is easily registered on PET/CT. Constant cytokine-mediated communication between Reed-Sternberg cells and inflammatory infiltrate is responsible for the maintenance and proliferation of Reed-Sternberg cells, but also for increased metabolic activity of the surrounding inflammatory infiltrate, and consequently increased FDG accumulation [11, 12]. Therefore, soon after starting chemotherapy and reducing the number of Reed-Sternberg cells, the metabolic activity of inflammatory cells decreases rapidly, while in patients resistant to initial chemotherapy, severe FDG avidity will still be detected on the PET/CT scan [13].

Choosing the optimal therapy based on PET/CT scan results in individuals initially diagnosed at an advanced stage of the disease is important from the aspect of sparing patients from unnecessary exposure to the toxicity of intense therapy modalities, which increases the likelihood of secondary malignancies. Gallamini et al. [14] compared the final therapy effect and the PFS duration in two groups of patients that were diagnosed at an advanced stage of the disease, but were given different initial treatment. The first group received de-escalation therapy according to the escalated bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone (eBEACOPP) protocol, while patients in the second group received a standard therapy with two ABVD cycles, after which therapy was escalated only in patients with positive interim PET/CT scan results. The authors reported no statistically significant differences in achieving and maintaining remission between these two groups, suggesting that it is not justified to expose patients to additional toxicity by routine use of eBEACOPP protocol, except when initial resistance to the first-line therapy is confirmed by PET/CT [14].

In our patients, CT was a more frequently adopted radiological method during the initial disease staging than PET or PET/CT (16 versus 6 patients, respectively). However, data reported in pertinent literature indicate that the assessment of FDG avidity on PET or PET/CT scans during the initial disease staging is superior to the assessment of lymph node size and the affection of extranodal structures on CT scans [13, 15]. In several prospective studies [16–25], as a part of which the results of these two radiological methods were compared in the same group of patients, the same specificity and significantly higher sensitivity of PET and PET/CT compared to classical contrast-enhanced CT was verified. The percentage of patients in these studies who were diagnosed at a higher clinical stage of the disease based on the PET or PET/CT scan findings ranged from 9% to 41% (18% on average), while the lower clinical stage of the disease compared to classical contrast-enhanced CT was verified in 0 - 12% of patients (5% on average). The main reason for the diagnosis at a higher clinical stage of the disease was the detection of extranodal lesions on the PET/CT scan, primarily in the bone marrow. These results are consistent with the fact that bone marrow involvement in Hodgkin's lymphoma is mostly focal and more difficult to detect on a pathohistological sample, while high FDG sensitivity of PET/CT scan makes it much easier to detect bone marrow lesions [3, 23]. Accordingly, many authors are questioning the utility of routine bone marrow biopsy if PET/CT is used during the initial diagnosis. Moreover, as their meta-analysis of nine studies including 955 subjects in total confirmed the 87.5 - 100% sensitivity and 86.7 - 100% specificity of PET/CT scan in detecting bone marrow lesions, Adams et al. concluded that routine bone marrow biopsy should be replaced by PET/CT scan at initial diagnosis [26]. Furthermore, international guidelines for treatment of FDG avid lymphomas recommend the use of PET/CT scan during the initial disease staging, while the justification for using classical CT is increasingly being questioned [27].

In the present study, none of the parameters included in the IPS showed to be statistically significant prognostic factors for PFS, countering the prevailing view that IPS is one of the main modalities for disease outcome assessment [28]. Although retrospective studies conducted at the end of 20th century confirmed that these seven factors are independently associated with more adverse disease outcomes, recent investigations suggest that the prognostic significance of IPS exists, but considering that advances in therapy mostly affect patients at the advanced stage of the disease, its predictive value is decreasing. Therefore, the relevance of the IPS in predicting disease outcome when using interim PET/CT scan and optimizing therapy based on the obtained findings is debatable [28–31].

These results in relation to the findings and conclusions yielded by other studies, as well as the fact that the main limitations of our study originate from the retrospective design and a small sample of patients, indicate the need for prospective studies involving a greater number of patients that would be followed over a longer period of time, since it would produce more accurate results.

Conclusion

Interim positron emission tomography/computed tomography is a major prognostic factor in predicting treatment response and disease outcome in patients with newly diagnosed Hodgkin's lymphoma.

Using the interim positron emission tomography/computed tomography and choosing the optimal therapeutic approach based on its results reduces the relevance of International Prognostic Score in predicting the disease outcome.

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CHARACTERISTICS OF ISCHEMIC STROKE IN PATIENTS WITH CONFIRMED CORONAVIRUS DISEASE 2019 INFECTION IN VOJVODINA, SERBIA

KARAKTERISTIKE ISHEMIJSKOG MOŽDANOG UDARA KOD PACIJENATA SA POTVRĐENIM COVID-19 U VOJVODINI, SRBIJA

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Summary

Introduction. The World Health Organization declared a pandemic of coronavirus disease 2019 on March 11, 2020. The literature has shown that coronavirus disease 2019 is associated with thromboembolic complications, including ischemic stroke. This retrospective study aims to present the characteristics of ischemic stroke in patients with coronavirus disease 2019. **Material and Methods.** This retrospective study was conducted in the period from April 2020 to January 2022 at the Clinical Center of Vojvodina, and included 58 patients with confirmed coronavirus disease 2019 and a new-onset ischemic stroke. **Results.** The reason for hospitalization in 42 (72.41%) patients was a new-onset ischemic stroke and 16 of them (38.09%) had a previously confirmed coronavirus disease 2019. In 16 patients (27.58%), ischemic stroke occurred during hospital treatment due to severe clinical manifestations of coronavirus disease 2019. In most coronavirus disease 2019 positive patients the etiology of ischemic stroke was unknown. In most cases (43.1%), the ischemic stroke was a partial infarction of the anterior circulation. Nine patients (15.51%) received intravenous thrombolytic therapy (alteplase) in the appropriate time frame. Lethal outcome occurred in 21 patients, of which in 11 patients complications of coronavirus disease 2019 were the cause of death. **Conclusion.** A large number of cases with stroke and thrombotic complications in patients with coronavirus disease 2019 is a good indicator that severe acute respiratory syndrome coronavirus 2 does not only cause respiratory infections, but is a systemic infection. The etiology of most ischemic strokes was unknown, which is probably related to difficult functioning of the health system during the pandemic.

Key words: Ischemic Stroke; COVID-19; SARS-CoV-2; Thromboembolism; Demography; Risk Factors; Treatment Outcome

Sažetak

Uvod. Svetska zdravstvena ogranizacija je 11. marta 2020. godine proglasila pandemiju koronavirusne bolesti 2019 (COVID-19). Podaci iz literature ukazuju na povezanost COVID-19 i tromboembolijskih komplikacija, uključujući ishemijski moždani udar. Cilj ove retrospektivne studije bio je da prikaže karakteristike ishemijskih moždanih udara kod pacijenata obolelih od COVID-19. **Materijal i metode.** Ova retrospektivna studija je sprovedena u Univerzitetskom kliničkom centru Vojvodine i obuhvatila je 58 pacijenata u periodu od aprila 2020. do januara 2021. godine koji su imali udružen COVID-19 sa novonastalim ishemijskim moždanim udarom. **Rezultati.** Razlog za hospitalizaciju kod 42 pacijenta (72,41%) bio je novonastali moždani udar, 16 od njih (38,09%) imalo je prethodno potvrđen COVID-19. Ishemijski moždani udar kod 16 pacijenata (27,85%) nastao je tokom hospitalnog lečenja zbog teške kliničke slike COVID-19. Najčešći etiopatogenski mehanizam nastanka ishemijskog moždanog udara kod COVID-19 pozitivnih pacijenata bio je nepoznate etiologije. U najvećem broju slučajeva (41,1%) ishemijski moždani udar je bio parcijali infarkt prednje cirkulacije. Devet pacijenata (15,51%) je primilo intravensku trombolitičku terapiju (alteplazu) u odgovarajućem "vremenskom prozoru". Smrtni ishod se desio kod 21 pacijenta, od kojih su kod 11 pacijenata uzroci smrti bile komplikacije COVID-19. **Zaključak.** Veliki broj udruženih slučajeva moždanog udara i trombotskih komplikacija kod COVID-19 pozitivnih pacijenata je pokazatelj da SARS-CoV-2 nije samo uzročnik respiratorne infekcije, nego sistemske infekcije. Etiologije većine ishemijskih moždanih udara je bila kriptogena, što je verovatno povezano sa otežanim funkcionisanjem zdravstvenog sistema u periodu pandemije.

Ključne reči: ishemijski moždani udar; COVID-19; SARS-CoV-2; tromboembolija; demografija; faktori rizika; ishod lečenja

Introduction

On March 11, 2020, the World Health Organization declared a pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). By October 2, 2022, 615 million cases were confirmed, with a mortality rate of 1.05%. In the Republic of Serbia, the total number of patients by October 4, 2022, was 2,369,456, and the mortality rate was 0.72% [1]. The severity of the clinical symptoms in patients with COVID-19 varies, from asymptomatic form of the disease, mild and more severe clinical presentation with bilateral pneumonia, to life-threatening acute respiratory distress syndrome and multiorgan dysfunction that can lead to death.

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Abbreviations

COVID-19	– coronavirus disease 2019
SARS-CoV-2	– severe acute respiratory syndrome coronavirus 2
ACE2	– angiotensin-converting enzyme 2
vWF	– von Willebrand factor
CT	– computed tomography
Ang	– angiotensin
IL	– interleukin
NIHSS	– National Institutes of Health Stroke Scale
TACI	– total anterior infarction
PACI	– partial anterior infarction
POCI	– posterior circulation infarction
LACI	– lacunar infarction
mRS	– modified Rankin Scale
TOAST	– Trial of (Org 10172) Acute Stroke Treatment

Neurological symptoms are common in COVID-19 positive patients, such as loss of the sense of smell and taste, headache, dizziness, and symptoms that are related to the development of ischemic stroke. The incidence of stroke in COVID-19 patients is estimated to be 0.5 - 1.5% [2]. In a retrospective study from the beginning of the pandemic that included 214 hospitalized COVID-19-positive patients from Wuhan, 5.7% of patients developed a stroke [3]. Hemorrhagic stroke is much less common than ischemic stroke in COVID-19 patients [4, 5].

Arterial and venous thrombosis

There is a lot of evidence of arterial and venous thrombotic complications in patients infected with COVID-19. In the study by Klok et al., 31% of COVID-19 patients had thrombotic complications, most often deep vein thrombosis, pulmonary thromboembolism, myocardial infarction, and ischemic stroke [6].

Autopsies of COVID-19 patients showed numerous micro- and macrovascular thrombosis of the blood vessels in the lungs, liver, spleen, kidneys, and heart. Acute respiratory insufficiency may be caused by microvascular thrombosis in the lungs [6]. Pulmonary thromboembolism is the cause of death in a large percentage of COVID-19-positive patients [7].

The exact cause of thrombotic complications has not been established, but it is considered to be multifactorial. One of the possible explanations for the pathophysiological mechanism of thrombotic complications in patients infected with COVID/19 is Virchow's triad [8].

Endothelial injury

The first factor of Virchow's triad is endothelial injury, which may be a result of direct effect of the virus on the cells or as part of a systemic inflammatory response - a cytokine storm [8]. Endothelial cell damage changes the vascular wall and causes platelet activation, and enhances procoagulant activity.

Direct endothelial cell damage

Coronavirus binds to the angiotensin-converting enzyme 2 (ACE2) which is widely expressed in multiple organs. The SARS-CoV-2 binds to ACE2 receptors with antigenic protein spikes ("spike" antigen) and enters the cell through processes involving the cell surface transmembrane protein serine 2 (TMPRSS2). The viral genome ribonuclric acid is released and replicates inside the host cell's cytoplasm. The newly formed genomic ribonucleic acid is packaged into vesicles containing the virion, fuses with the cell membrane, and thus releases the virus. This process enables the circulation of the virus. The ACE2 receptors are expressed widely in multiple organs, including the lungs, heart, kidneys, and intestines [9]. Underneath the endothelial cells is von Willebrand factor (vWF) coagulation, which is released after endothelial cell damage. The vWF plays a role in platelet adhesion and binds to factor VIII in the blood, thus prolonging its half-life, and also plays a role in starting the coagulation cascade [10].

Cytokine storm

Cytokine storm, as a complication of COVID-19, is an uncontrolled response to SARS-CoV-2 infection with the synthesis of interleukin (IL)-6 and other inflammatory mediators. This condition damages cells, produces numerous pro-inflammatory markers, and initiates coagulation process via the complement system, and thus induces a state of hypercoagulability. As part of COVID-19, an increase in cytokine values was recorded, including IL1B, IFNγ, IP10, MCP1, and IL-6, especially in patients with a more severe form of the disease and with higher mortality [9].

Dysregulation of the renin-angiotensin system

Dysregulation of the renin-angiotensin system is also a cause of cell damage and thrombotic complications. The virus invades alveolar epithelium and cardiomyocytes using ACE2 as a transmembrane receptor. The ACE2 receptors are part of the renin-angiotensin system that participates in the control of arterial pressure. The ACE2 is a counter-regulatory peptide that degrades angiotensin (Ang) II into Ang 1 - 7, thereby attenuating the biological effects of the AT1 receptor. Angiotensin 1 - 7 is a protective peptide and has vasodilatory and anti-inflammatory effects. Depletion of ACE2 receptors stops the enzymatic conversion of Ang II to Ang 1 - 7. A deficiency of ACE2 reduces the production of Ang 1 - 7 and leads to disruption of endothelial function and increases oxidative stress. The hormone Ang II leads to an increase in blood pressure and has a pro-inflammatory and atherogenic effect on blood vessels that causes lung damage and damages organs such as the heart and brain. An elevated level of the Ang II hormone was registered in 90% of critically ill patients with COVID-19 [9].

Hypercoagulability

The hypercoagulable state is one of the most significant indicators of a poor outcome in COVID-19 with high D-dimer values and decreased platelet values as a consequence of consumption (disseminated) coagulopathy. In some patients with confirmed COVID-19, elevated values of antiphospholipid antibodies (anticardiolipin and anti-beta-2 glycoprotein antibodies) were observed, which can lead to thrombotic events.

Transiently elevated antiphospholipid antibodies have been observed in critically ill patients [10].

Stasis of blood flow

The last factor of Virchow's triad is blood stasis, which occurs as a result of the immobility of COVID-19 patients due to their poor general condition and dependence on oxygen support [8]. Because of that, it is necessary to include patients in early rehabilitation treatment.

Cardioembolism

Cardiomyopathy in COVID-19 patients can be explained by several mechanisms, such as a direct effect of virus particles via ACE2 receptors, systemic inflammatory reaction of the body (cytokine storm), a reduction of ACE2 receptor regulation and increased effect of the hormone Ang II, as well as hypoxemia and respiratory insufficiency as a consequence of lung tissue damage. All these contribute to the development of myocarditis and heart rhythm disorders, which increases the risk of cardioembolic stroke [11].

Material and Methods

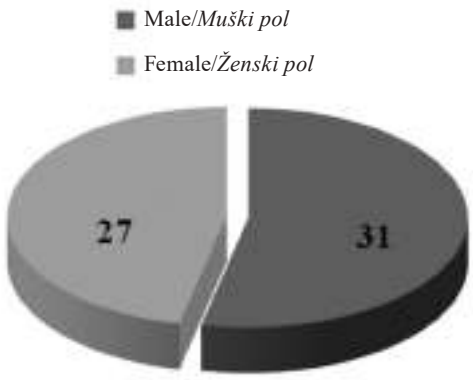
This retrospective study was conducted at the University Clinical Center of Vojvodina, and it included all patients treated from April 2020 to January 2022, who required treatment in a tertiary healthcare institution. Patients had a COVID-19 confirmed by rapid antigen or PCR testing and ischemic stroke.

We studied data related to the demographic and clinical characteristics of patients and the characteristics of ischemic stroke. The National Institutes of Health Stroke Scale (NIHSS) was used for clinical assessment. Based on the clinical manifestations and radiological findings, ischemic strokes were classified into one of four possible types based on the Oxfordshire Community Stroke Project (OCSP) classification (total anterior infarction (TACI), partial anterior infarction (PACI), posterior infarction (POCI), and lacunar infarction (LACI). The neurological outcomes of patients were based on modified Rankin Scale (mRS). We discussed the etiology of ischemic stroke based on the Trial of Org Acute Stroke Treatment (TOAST) classification.

Results

The retrospective study included groups of patients who were hospitalized at the University Clinical Center of Vojvodina with confirmed COVID-19 and ischemic stroke from April 2020 to January 2022. The COVID-19 was confirmed by rapid antigen or PCR testing for SARS-CoV-2. Patients who were infected with COVID-19 during hospital treatment for a stroke were excluded from the study.

By searching the Key Information Summary of the University Clinical Center of Vojvodina, a group of 58 patients was selected, of which 31 were male and 27 were female **(Graph 1).**



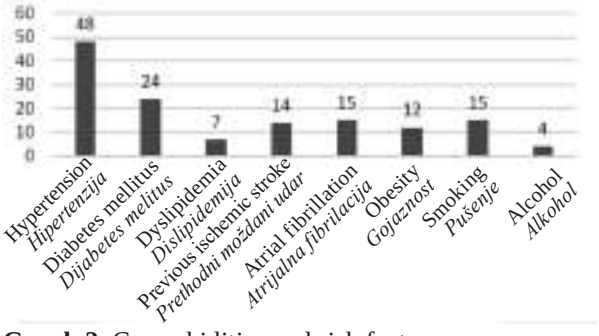
Graph 1. Gender distriburion of patients
Grafikon 1. *Struktura pacijenata po polu*

The average age of the examined patients was 77 years, of which twelve patients were over 80 years old, and nine patients were under 65 years old. The youngest patient was 35 years old with no previous comorbidities.

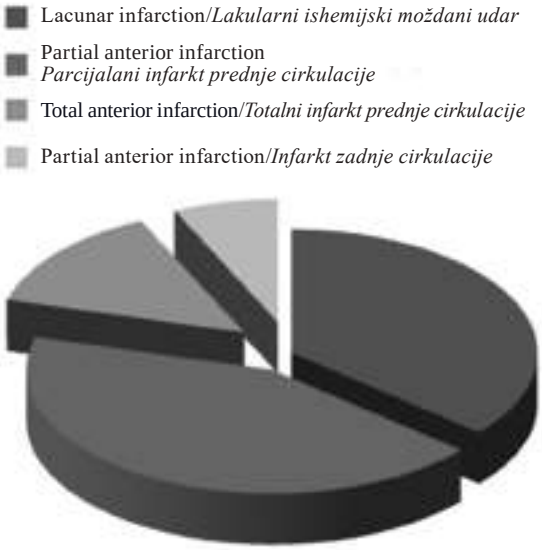
In 42 (72.41%) patients the reason for admission was a new-onset ischemic stroke and 16 (38.09%) had a previously confirmed COVID-19. The median time between the COVID-19 symptoms and stroke onset was 11 days (interquartile range: 4 - 15). In 26 (62%) patients, COVID-19 was confirmed by PCR or antigen test after admission due to new-onset ischemic stroke.

Ischemic stroke occurred in 16 patients (27.58%) during hospital treatment due to the severe clinical picture of COVID-19. In the first week of COVID-19 treatment, stroke occurred in 5 (33.33%) patients, in 4 (26.66%) patients in the second, and in 7 (43.75%) patients in the third week.

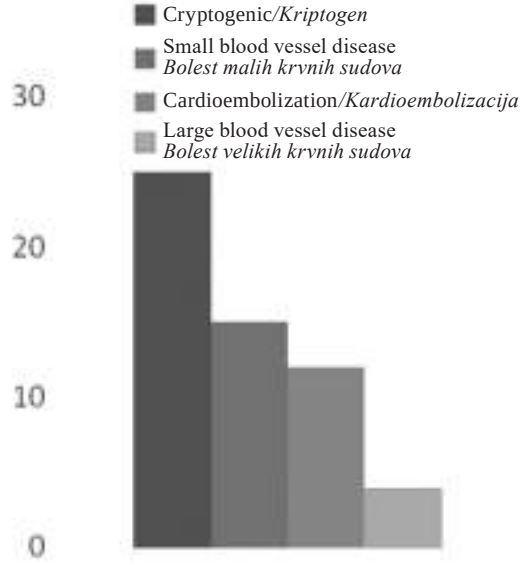
There were 4 patients (6.89%) without previously known comorbidities, while the other patients had at least one risk factor for ischemic stroke. The most prevalent risk factors for stroke were hypertension (82.75%), diabetes mellitus (41.37%), atrial fibrillation (25.86%), obesity (18.96%), and dyslipidemia (12.06%). Previous stroke was reported in 14 patients (24.13%). Fifteen patients (25.86%) reported harmful habits such as cigarette smoking, and 4 patients (6.89%) reported large quantities of alcohol consumption **(Graph 2).** Premorbid mRS in 9 patients (15.51%) was ≥ 3.



Graph 2. Comorbidities and risk factors
Grafikon 2. *Komorbiditeti i faktori rizika*



Graph 3. Oxfordshire Community Stroke Project classification
Grafikon 3. *Projekt Oksfordširske zajednice za klasifikaciju moždanog udara*



Graph 4. Trial of (Org 10172) Acute Stroke Treatment - classification
Grafikon 4. *Ispitivanje (Org 10172) lečenja akutnog moždanog udara – klasifikacija*

According to the Oxfordshire Community Stroke Project classification of ischemic strokes, TACI was found in 8 patients (13.79%) and PACI in 25 patients (43.1%). Only 4 patients (6.89%) had a POCI, and in 21 patients (36.20%) LACI was verified by brain computed tomography (CT) **(Graph 3).**

In the largest number of patients (43.1%), the etiopathogenic mechanism of ischemic stroke was cryptogenic. Other types of ischemic strokes according to the TOAST classification were results of small blood vessel disease (25.86%), cardioembolization (20.68%), and large blood vessel disease (10.34%) **(Graph 4).** The median NIHSS was 10 (interquartile range: 8 - 14).

The highest prevalence of ischemic stroke of unknown etiology was probably due to different functioning of the tertiary healthcare system during the COVID-19 pandemic. During hospitalization, 33 patients (56.89%) had no complete diagnostic workup for stroke (arterial duplex scan, CT/magnetic resonance (MR) angiography, and cardiological examinations).

Nine patients (15.51%) received intravenous thrombolytic therapy (alteplase) in the appropriate time frame, of which seven patients (77.7%) had a favorable outcome. Signs of hemorrhagic transformation after intravenous thrombolytic therapy were not registered. No endovascular thrombectomy was performed. During hospitalization, all examined patients were treated by anticoagulant therapy with low-molecular-weight heparin and antiplatelet therapy.

All patients had chest X-rays, and 45 (77.58%) had bilateral pneumonia. All of them needed oxygen support via a mask. In six patients (10.34%), respiratory insufficiency escalated and they required mechanical ventilation.

Almost all examined patients (86.20%) had elevated of C-reactive protein (CRP) values, only 4 patients (6.89%) had D-dimer values within the reference range, while 10 patients (17.24%) had elevated D-dimer values > 10,000 ng/L. Six patients (10.34%) had elevated values of IL-6 during hospitalization.

Eight patients (13.79%) were transferred to another health facility for further hospital treatment and rehabilitation. Twenty-nine (50%) patients were referred for further home treatment with recommendations for controls in the Covid clinic. Thirty-nine (67.24%) patients had a severe disability at discharge, mRS $\geq$ 4. There were 21 (36.2%) lethal outcomes, of which the cause of death in eleven patients were complications of COVID-19. The average length of hospitalization in the examined patients was 16 days.

Discussion

Our retrospective study focused on studying the characteristics and subtypes of ischemic stroke in patients with confirmed COVID-19, as well as the disease outcomes at the end of hospitalization. The average age of patients was high, 77 years, with a predominance of men, in contrast to the study by Shahjouei et al., where 36% of patients were under 55 years of age, and 46% were under 65 years of age [12]. In our study, only 15.51% of patients were under 65 years of age, and the youngest patient was 35 years old with no previous risk factors for stroke. The reason for hospitalization in three-quarters of our patients was a new-onset ischemic stroke, of which 62% had asymptomatic COVID-19, which is comparable to the data from the study by Shahjouei et al. [12]. Some studies have shown that in addition to traditional cerebrovascular risk factors, an associated risk factor in patients with COVID-19 includes hypercoagulability [13]. Our study showed a high prev-

alence of known risk factors in ischemic stroke patients. Only four patients did not have at least one of the previously known comorbidities and risk factors for ischemic stroke. It was established that coagulopathies are often associated with elevated D-dimer values. In our study, only 4 (6.89%) patients did not have elevated D-dimer values, while 17.24% of patients had elevated values > 10,000.

Based on the TOAST classification, the largest number of patients did not have a clearly defined mechanism for ischemic stroke at the end of hospitalization. In less than half of cases (43.1%), the cause of ischemic strokes was not established, but it was assumed to be related to thrombotic complications as part of COVID-19. A possible cause of the large prevalence of cryptogenic stroke may be associated with the difficult functioning of the health system during the epidemic, the short time of hospitalization, and the impossibility of conducting all necessary neurosonological and neuroimaging diagnostic methods. In our study, 56.89% of patients did not complete neuroserological and neuroimaging diagnostics, as well as cardiological examinations during hospitalization. Two retrospective observational studies from New York showed that the highest percentage were cryptogenic strokes [14, 15]. Small vessel disease was the etiological mechanism in 25.86% of our patients, while neuroimaging analysis indicated LACI in 35.20% of patients. These rates are supported by worldwide studies based on the general population unrelated to COVID-19, small vessel infarction in 21% - 44% and LACI 21 - 30% of patients [16, 17].

In previous studies of COVID-19-positive patients, the incidence of small vessel infarction was 12 - 31%, which coincides with our results [18]. In our study, the lowest incidence was due to large blood vessel disease (10.34%), which can be related to the highest percentage of cryptogenic strokes. The highest incidence of large vessel disease as an etiological mechanism of ischemic stroke was in patients with associated COVID-19, with lower rates of small vessel disease and LACI in most studies [19, 20].

The fact is that LACI and diseases of small blood vessels have smaller deficits and disability [21–23]. During the pandemic, patients with mild to moderate symptoms were less likely to come to medical centers and thus remained less recognized, which could be related to the lower incidence of small blood vessel disease in the examined patients [24].

In our study, 15.51% of patients were treated with intravenous thrombolytic therapy. These rates are similar to a multinational study including 174 infected patients (12.7% intravenous thrombolysis, 5.2% mechanical thrombectomy) [25]. Mechanical thrombectomy was not performed on our patients. A study from Birmingham, as well as a study from New York, showed a significantly worse outcome at the end of hospitalization (NIHSS score) in patients with ischemic stroke associated with COVID-19 than in the population without COVID-19 (NIHSS 19 [23] vs. 8 [12]) [15, 26].

According to a large multihospital retrospective observational study from the United States of America, COVID-19-positive patients had more severe strokes with a worse outcome, 33.3% of patients died [14]. Our study showed similar result and the number of fatal outcomes was 36.2%. Other studies, including colleagues from Italy and New York, have also reported worse outcomes in patients with ischemic stroke associated with COVID-19 [15, 25]. An increase in inflammatory markers was noted in the majority of patients (86.2%) in our study, as well as in other studies worldwide [13–15, 27].

Our study has several limitations, but they may raise ideas for further research. First, this is a retrospective observational study with a small sample size that limits multivariable statistical analyses. Second, not all patients had the same completeness of laboratory data, so some comparisons could not be made. Third, our study did not compare cohorts of ischemic stroke patients with and without COVID-19. Fourth, we do not have all the outcomes at the end of hospitalization, since some patients were sent for further inpatient rehabilitation treatment or transferred to another health facility. Fifth, we did not study hemorrhagic stroke as a subtype.

Conclusion

The coronavirus disease 2019 pandemic, as the consequence of the systemic severe acute respiratory syndrome coronavirus 2 infection, has left an indelible mark on the quality of health care in all parts of the world. Stroke, as the leading cause of disability, is a major burden on the healthcare systems. The large number of strokes and other thrombotic complications in symptomatic and asymptomatic patients with coronavirus disease 2019 is a good indicator that severe acute respiratory syndrome coronavirus 2 is not only the cause of respiratory infections but a systemic infection. In our study, a lower percentage of large blood vessel disease, as an etiological factor of ischemic stroke, was recorded compared to other studies worldwide. A high rate of asymptomatic coronavirus disease 2019 at the time of stroke was also observed. Coronavirus disease 2019 has also caused damage to patients suffering from other chronic non-communicable diseases in terms of the impossibility of regular controls and elective surgeries due to the burden on the health system and insufficient capacities of health institutions.

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CASE REPORTS
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INTRAOPERATIVE CELL SALVAGE IN THE TREATMENT OF WUNDERLICH SYNDROME CAUSED BY RENAL ANGIOMYOLIPOMA

ULOGA APARATA ZA INTRAOPERATIVNU AUTOTRANSFUZIJU U LEČENJU VUNDERLIHOVOG SINDROMA UZROKOVANOG BUBREŽNIM ANGIOMIOLIPOMOM

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Summary

Introduction. Wunderlich syndrome is defined as acute spontaneous hemorrhage into the subcapsular and perirenal spaces in the absence of trauma. The most common causes of Wunderlich syndrome are various renal neoplasms, polycystic and vascular kidney diseases. Wunderlich syndrome is common in patients who use anticoagulant therapy or suffer from various types of congenital coagulopathies. The clinical diagnosis is usually confirmed by computer tomography of the abdomen and requires urgent surgical treatment or renal artery embolization. **Case Report.** A 45-year-old woman was admitted to the Emergency Center due to acute pain in the left abdomen, associated with arterial hypotension. A computer tomography of the abdomen was performed using intravenous contrast, which showed a ruptured tumor of the left kidney with a massive retroperitoneal hematoma. The tumor was 7 cm in diameter and according to the computer tomography data it corresponded to an angiomyolipoma. The patient underwent emergency surgery. Intraoperative cell salvage was used with additional blood replacement. Nephrectomy and drainage of the massive retroperitoneal hematoma were performed. Pathohistological analysis of the surgically removed kidney confirmed a renal angiomyolipoma. **Conclusion.** Wunderlich syndrome caused by the rupture of angiomyolipoma is still a life-threatening condition that should be considered in the differential diagnosis of sudden abdominal pain, especially in women of reproductive age. In hypotensive patients, available radiological methods should be used to make an accurate diagnosis, before taking the patient to the operating room.

Key words: Angiomyolipoma; Kidney Neoplasms; Retroperitoneal Space; Hemorrhage; Signs and Symptoms; Diagnosis; Blood Transfusion, Autologous; Nephrectomy

Sažetak

Uvod. Vunderlihov sindrom je definisan kao akutno nastalo spontano krvarenje u supkapsularnom i perirenalnom prostoru u odsustvu traume. Najčešći uzroci Vunderlihovog sindroma su različiti tumori bubrega, policistična i vaskularne bolesti bubrega. Vunderlihov sindrom je češći kod pacijenata koji koriste antikoagulacionu terapiju ili boluju od različitih vrsta kongenitalnih koagulopatija. Klinički postavljena dijagnoza se obično potvrđuje komjuterizovanom tomografijom abdomena i zahteva hitno hirurško lečenje ili embolizaciju renalne arterije. **Prikaz slučaja.** Žena starosti 45 godina je primljena u Urgentni centar zbog akutnog bola u levoj polovini trbuha koji je praćen arterijskom hipotenzijom. Načinjena je kompjuterska tomografija abdomena sa intravenskim kontrastom koji je pokazao postojanje rupturiranog tumora levog bubrega sa masivnim retroperitonealnim hematomom. Tumor je bio prečnika 7 cm i prema tomografskim karakteristikama je odgovarao angiomiolipomu. Pacijentkinja je hitno operisana. Primenjen je aparat za intraoperativnu autotransfuziju uz dodatnu nadoknadu krvi. Urađena je nefrektomija i drenaža masivnog retroperitonealnog hematoma. Patohistološkom analizom hirurški odstranjenog bubrega potvrđeno je da se radi o angiomiolipomu bubrega. **Zaključak.** Vunderlihov sindrom uzrokovan rupturom angiomiolipoma i dalje predstavlja životno ugrožavajuće stanje koje treba uzeti u obzir u diferencijalnoj dijagnozi iznenadno nastalog abdominalnog bola, posebno kod žena u reproduktivnom periodu života. Kod hipotenzivnih pacijenata treba koristiti dostupne radiološke metode radi postavljanja tačne dijagnoze, pre uvođenja pacijenta u operacionu salu.

Ključne reči: angiomiolipom; tumori bubrega; retroperitonealni prostor; krvarenja; znaci i simptomi; dijagnoza; autologna transfuzija; nefrektomija

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Abbreviations
WS – Wunderlich syndrome
CT – computed tomography

Introduction

Wunderlich syndrome (WS) is defined as acute spontaneous hemorrhage into the subcapsular and perirenal kidney spaces in the absence of trauma. This syndrome was first described in 1856. The WS is characterized by Lenk's triad: acute flank or abdominal pain, a palpable flank mass, and hypovolemic shock [1]. Nontraumatic retroperitoneal hemorrhage is commonly caused by rupture of an abdominal aortic aneurysm, adrenal bleeding, or coagulopathy. The most common causes of WS are various renal neoplasms, especially angiomyolipoma and renal cell carcinoma, but also cystic and vascular kidney diseases. The WS is common in patients who use anticoagulant therapy or suffer from various types of congenital coagulopathies.

Clinically established diagnosis is usually confirmed by computed tomography (CT) of the abdomen and requires urgent surgical treatment or renal artery embolization. The choice of treatment depends on the severity of the clinical presentation as well as the cause itself. Surgical treatment includes total or partial nephrectomy. Total nephrectomy is the treatment of choice in cases when bleeding is caused by a spontaneous rupture of renal cell carcinoma or angiomyolipoma larger than 5 cm in diameter.

Case Report

A 45-year-old woman was admitted to the Emergency Center due to acute pain in the left hemiabdomen, associated with arterial hypotension. The patient was communicative during admission. Sudden pain in the left abdomen occurred about an hour before admission. She complained of weakness and dizziness and denied injuries to the body before the pain started. During the physical examination, there was no evidence of injuries. The patient had no chronic diseases. She underwent an umbilical hernia surgery two weeks before admission and had no other prior surgeries.

Abdominal CT was performed immediately using intravenous contrast. It showed a ruptured left kidney tumor with massive retroperitoneal hematoma. The largest diameter of the tumor was 7 cm and according to CT data it corresponded to an angiomyolipoma. Hematoma dimensions were 80 x 80 x 170 mm (anterior-posterior x latero-lateral x cranio-caudal), and density 50 - 60 Hounsfield units. The described hematoma was compressing the pancreas, stomach, small intestine and vascular pedicles of the kidney and spleen **(Figures 1, 2 and 3)**. The laboratory tests showed significant anemia. Due to hemodynamic instability, the patient underwent emergency surgery. Since the CT showed a renal angiomyolipoma and the patient lost a significant amount of blood, intraoperative cell salvage was per-

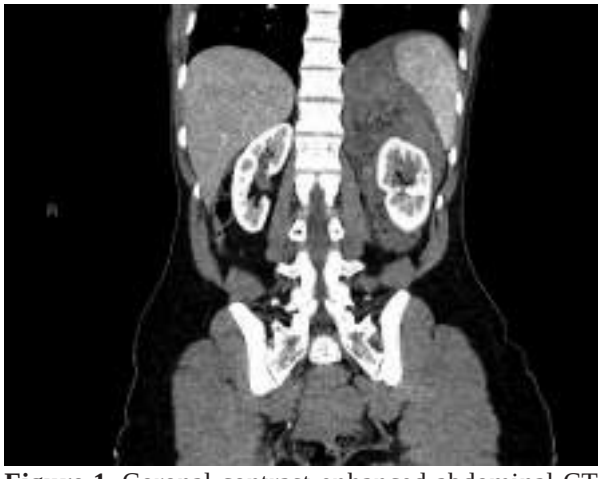

Figure 1. Coronal contrast-enhanced abdominal CT showing a massive left perirenal hematoma
Slika 1. *Koronalni kontrasni snimak kompjuterizovane tomografije abdomena pokazuje masivni levi perirenalni hematom*

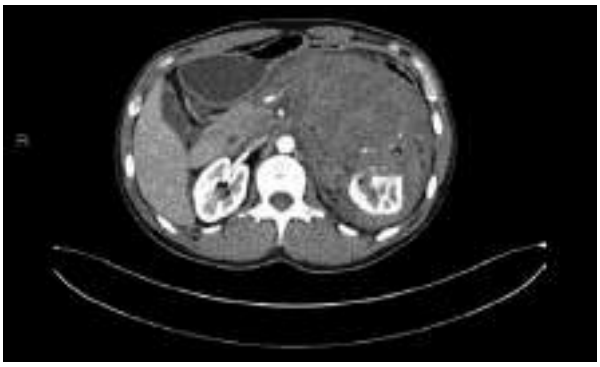

Figure 2. Abdominal CT – adipose tissue in the tumor indicates an angiomyolipoma
Slika 2. *Kompjuterizovana tomografija abdomena – adipozna komponenta tumora ukazuje na angiomiolipom*

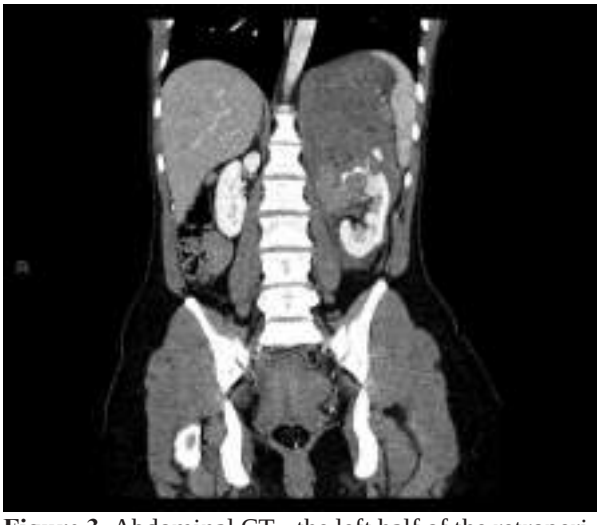

Figure 3. Abdominal CT - the left half of the retroperitoneum is filled with a massive hematoma spreading from the tumor mass in the upper pole of the left kidney
Slika 3. *Kompjuterizovana tomografija abdomena: leva polovina retroperitoneuma je ispunjena masivnim hematomom koji se pruža od gornjeg pola levog bubrega*

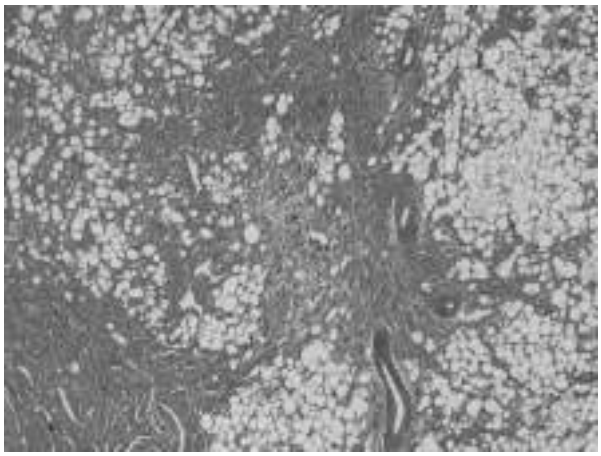

Figure 4. Angiomyolipoma (HE staining, 25x)
Slika 4. *Angiomiolipom HE bojenje, 25x*

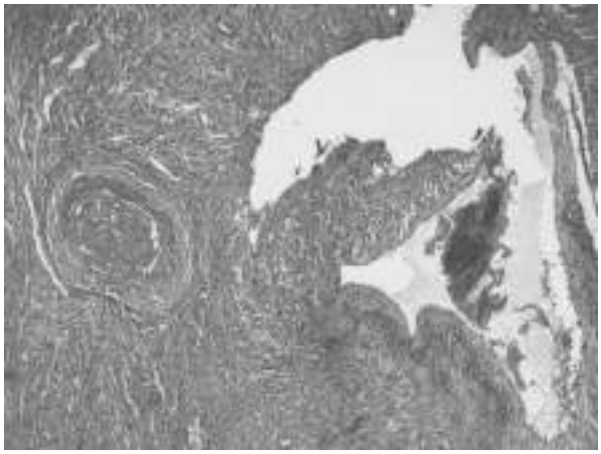

Figure 5. Angiomyolipoma, focal necrosis (HE stain, 50x)
Slika 5. *Angiomiolipom, fokalna nekroza, HE bojenje, 50x*

formed with additional blood replacement by resuspended erythrocytes and blood plasma. Transperitoneal approach to the left kidney was performed with a left subcostal incision. The renal artery and vein were ligated and cut, after which a nephrectomy and drainage of the massive retroperitoneal hematoma were performed. The total blood loss was about 2500 ml. By using a cell salvage device, 250 ml of red cells concentrate was saved and returned to circulation. Postoperatively, the patient was transferred to the intensive care unit. The recovery proceeded without complications. The patient was discharged on the tenth postoperative day and was followed up postoperatively; she had no complaints and the renal function was regular.

Pathohistological analysis of the surgically removed kidney confirmed a renal angiomyolipoma with the following immunohistochemical characteristics: HMB45+, MelanA+, STAT6-, CD34+, ER-, PR-, Calponin+, SMA+/-, p63-, GATA3-, RCC- **(Figures 4 and 5)**.

Discussion

The WS is a type of non-traumatic retroperitoneal hemorrhage. Massive retroperitoneal hemorrhage originating from the kidneys is a life-threatening condition that mainly requires urgent surgical treatment. The main risk factors for spontaneous rupture of angiomyolipoma are the size of the tumor, quantity and size of aneurysms in the tumor, pregnancy, as well as tuberous sclerosis.

In cases of spontaneous rupture of renal tumors, especially in patients who had not previously been diagnosed with renal tumors, radiological diagnosis plays a key role. The radiological method of choice is abdominal CT, even though at the time of hemorrhage its sensitivity is less than 60% [2].

Total nephrectomy is a preferable method of treatment if the cause of bleeding is a spontaneous rupture of renal cell carcinoma or angiomyolipoma larger than 5 cm in diameter. On the other hand, both traumatic and spontaneous retroperitoneal hemorrhage of renal origin can be treated conservatively, if the mechanism of occurrence and bleeding dynamic allows it (estimated by CT or magnetic resonance imaging). In cases of spontaneous rupture of a kidney tumor, radiological studies sometimes do not show the exact location of bleeding or the pathohistological type of the tumor, so surgical treatment is also a diagnostic procedure. The surgically removed tissue examined by a pathologist sets the final diagnosis and determines the further course of treatment and follow up of the patient. Cubillana et al. found that conservative management of WS represents a safe and acceptable treatment option in patients without malignant kidney tumors [3]. Sotosek et al. have successfully conservatively treated WS caused by spontaneous rupture of bilateral renal angiomyolipomas [4]. On the other hand, surgical approach is often necessary for tumors larger than 4 cm in diameter [5].

In our case, we performed a surgical treatment with total nephrectomy, due to the hemodynamic instability of the patient, who could not be treated conservatively. We decided to perform a total nephrectomy instead of a partial nephrectomy due to the size of the tumor, the extension of the tumor in the hilus of the kidney, as well as the existence of a functional contralateral kidney without pathological changes. Taking into account the massive bleeding as well as the findings of CT, which described a rupture of an angiomyolipoma and not a renal cell carcinoma, an intraoperative cell salvage device was used in order to save as much blood cells as possible. Cell salvage devices have been used for more than forty years in surgical treatment of various diseases [6]. A review of the existing literature did not reveal any previous intraoperative use of a cell salvage device in the management of WS. The patient also received fresh frozen blood plasma and resuspended erythrocytes intraoperatively.

Although angiomyolipoma is the most common benign kidney tumor, its prevalence is less than 0.15% in the general population. It is known that it is more common in women [7]. In most cases, patients with angiomyolipoma do not have symptoms and the tumor is mostly discovered incidentally using various radiological methods, primarily during examination of the abdomen and retroperitoneum with ultrasound, CT or magnetic resonance [8]. Low prevalence of this tumor in the population is the main reason why its rupture is

a relatively rare cause of sudden pain in the abdomen or retroperitoneum.

Conclusion

Wunderlich syndrome caused by rupture of angiomyolipoma is still a life-threatening condition that should be considered in the differential diagnosis of sudden abdominal pain. Spontaneous rupture of angiomyolipoma deserves its place in the differential diagnosis of sudden pain, especially in the population of fertile women. Available radiological methods should be used to make an accurate diagnosis in hypotensive patients before taking the patient to the operating room.

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BASILAR ARTERY OCCLUSION TREATED WITH MECHANICAL THROMBECTOMY IN EXTENDED TIME WINDOW USING DIFFUSION-WEIGHTED IMAGING/FLUID ATTENUATED INVERSION RECOVERY MISMATCH – A CASE REPORT

OKLUZIJA BAZILARNE ARTERIJE LEČENE MEHANIČKOM TROMBEKTOMIJOM U PRODUŽENOM VREMENSKOM PROZORU KORIŠĆENJEM DIFUZNOG TENZORSKOG IMIDŽINGA/FLUIDNO ANTENUIRANE INVERZIJSKE NEPODUDARNOSTI OPORAVKA – PRIKAZ SLUČAJA

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Summary

Introduction. There are individual case reports and case-series in the literature that have applied diffusion-weighted imaging/fluid attenuated inversion recovery mismatch and intravenous thrombolytic therapy in the treatment of posterior circulation strokes. This case report demonstrates the use of diffusion-weighted imaging/fluid attenuated inversion recovery mismatch in the treatment of basilar artery occlusion with mechanical thrombectomy. **Case Report**. A 68-year-old male patient presented with a wake-up stroke and a National Institutes of Health Stroke Scale score of 14. Computed tomography angiography showed an occlusion of the basilar artery. Diffusion-weighted imaging/fluid attenuated inversion recovery mismatch was established and mechanical thrombectomy was performed. Complete reperfusion was achieved. Mechanical thrombectomy was performed in the 16th hour from the onset of symptoms. After the intervention, the patient's National Institutes of Health Stroke Scale score was 9. The patient was discharged without any neurological symptoms and a score of 0 on the modified Rankin Scale. **Conclusion.** Diffusion-weighted imaging/fluid attenuated inversion recovery mismatch may be a useful criterion for the selection of patients with basilar artery occlusion and unknown-onset strokes who are to be treated with mechanical thrombectomy.

Key words: Stroke; Basilar Artery; Arterial Occlusive Diseases; Thrombectomy; Diffusion Magnetic Resonance Imaging; Predictive Value of Tests

Sažetak

Uvod. U literaturi postoje individualni prikazi slučaja i serije slučaja koji su primenjivali difuzioni tenzorski imidžing/fluidno-atenuiranu inverzijsku nepodudarnost oporavka i intravensku trombolitičku terapiju u lečenju moždanog udara zadnje cirkulacije. Ovaj prikaz slučaja demonstrira primenu difuzionog tenzorskog imidžinga/fluidno-atenuiranu inverzijsku nepodudarnost oporavka lečenju okluzije bazilarne arterije uz pomoć mehaničke trombektomije. **Prikaz slučaja**. Muškarac, starosti 68 godina, imao je moždani udar pri buđenju, sa skorom 14 na Skali za moždani udar Nacionalnog instituta za zdravlje. Kompjuterizovana tomografska angiografija je pokazala okluziju bazilarne arterije. Utvrđeno je postojanje difuzionog tenzorskog imidžinga/fluidno-atenuirana inverzijska nepodudarnost oporavka i sprovedena je mehanička trombektomija. Postignuta je potpuna rekanalizacija. Mehanička trombektomija je načinjena u 16. satu od početka simptoma. Nakon intervencije, skor na Skali za moždani udar Nacionalnog instituta za zdravlje bio JE 9. Pacijent je otpušten bez neuroloških simptoma; na modifikovanoj Rankinovoj skali. **Zaključak**. Difuzioni tenzorski imidžing/fluidno-atenuirana inverzijska nepodudarnost oporavka može biti koristan kriterijum za selekciju pacijenata sa okluzijom bazilarne arterije i nepoznatim vremenom moždanog udara, koji bi bili lečeni mehaničkom trombektomijom.

Ključne reči: moždani udar; bazilarna arterija; arterijske okluzivne bolesti; trombektomija; difuziona magnetna rezonanca; prediktivna vrednost testova

Introduction

Occlusion of the basilar artery (BA) and brainstem strokes have a high rate of morbidity and mortality [1]. Nowadays, there are confirmatory trials pointing how to identify patients with wake-up stroke eligible for thrombolysis using magnetic resonance imaging (MRI) [2] and patients eligible for mechanical thrombectomy in extended time window using perfusion imaging [3, 4], but all trials were done including only anterior circulation. We present a case of a patient with BA occlusion and wake-up stroke treated with mechanical thrombectomy (MT) along with diffusion-weighted imaging (DWI)/fluid attenuated inversion recovery (FLAIR) mismatch.

Case Report

A 68-year-old male patient was admitted to the Emergency Department with wake-up stroke 10 hours

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Abbreviations

BA	– basilar artery
MRI	– magnetic resonance imaging
MT	– mechanical thrombectomy
DWI	– diffusion-weighted imaging
FLAIR	– fluid attenuated inversion recovery
NIHSS	– National Institutes of Health Stroke Scale
CT	– computed tomography
CTA	– computed tomography angiography

after he was last seen well. The patient had posterior circulation symptoms, National Institutes of Health Stroke Scale (NIHSS) 14, Glasgow coma scale 12. Hyperdense sign of basilar artery was seen on the non-enhanced computed tomography (CT) **(Figure 1a)**,

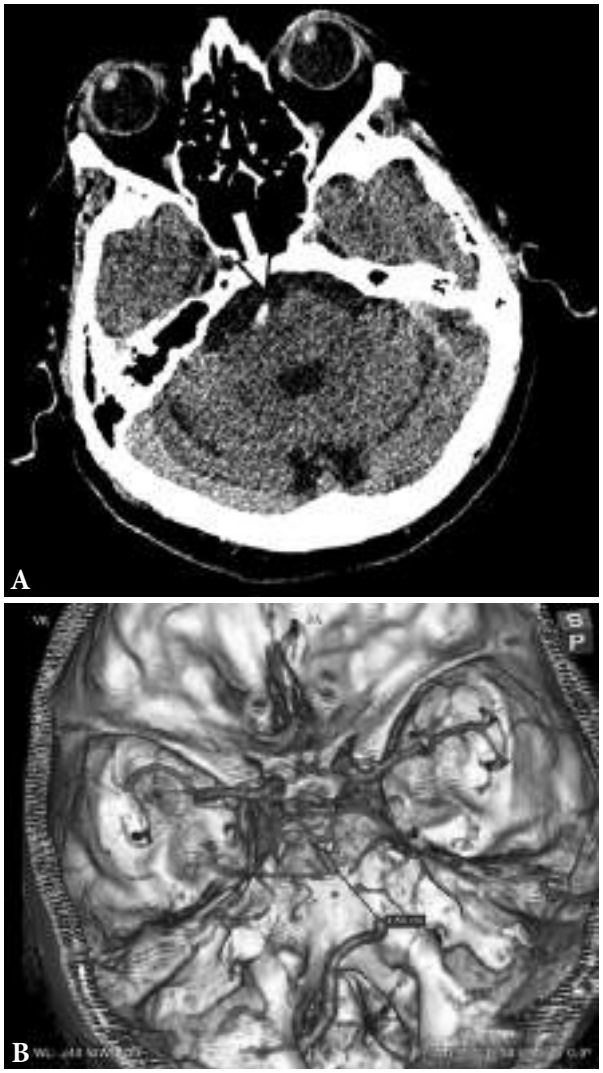

Figure 1. A) Non-contrast CT - hyperdense sign of basilar artery (arrow); B) CTA 3D reconstruction showing the basilar artery occlusion of 1.6 cm in length

Slika 1. *A) Nativna kompjuterizovana tomografija – Hiperdenzni znak bazilarne arterije (strelica); B) 3D rekonstrukcija kompjuterizovane tomografske angiografije sa mestom okluzije bazilarne arterije u dužini od 1,6 cm*

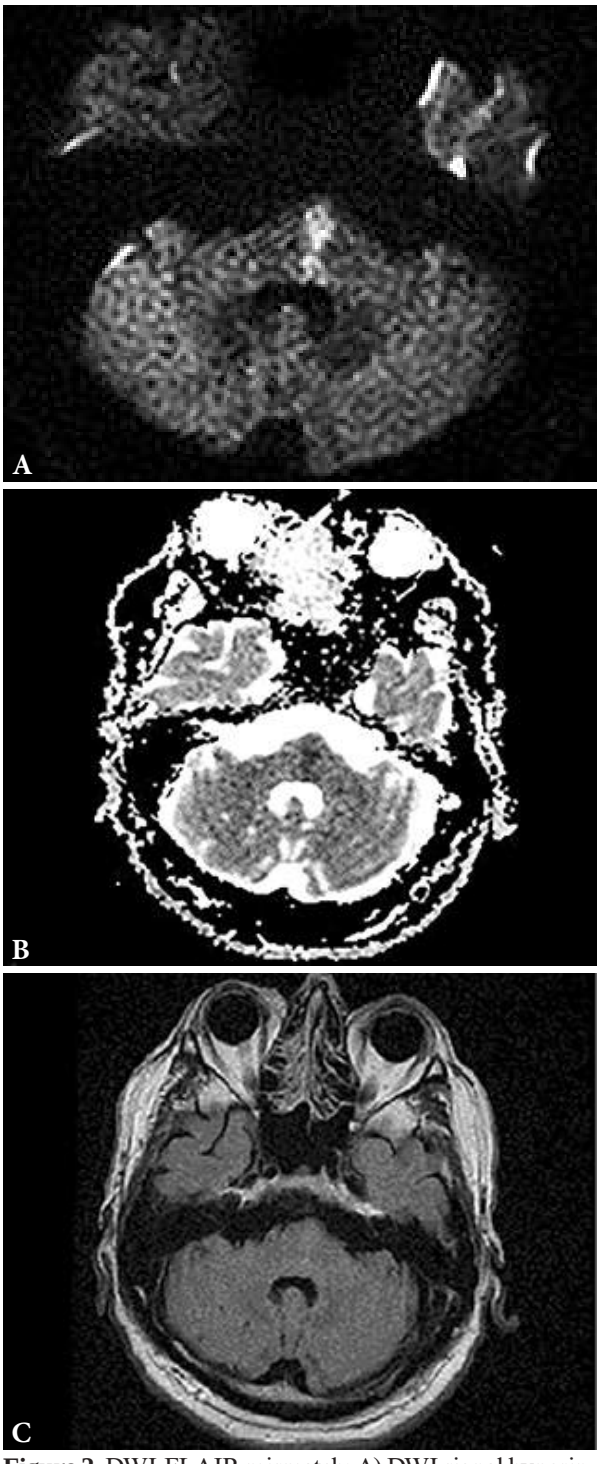

Figure 2. DWI-FLAIR mismatch: A) DWI signal hyperintensity in the left side of pons; B) Apparent diffusion coefficient map with a corresponding hypointense pontine zone; C) FLAIR without a hyperintense signal in the pons region

Slika 2. *DWI-FLAIR nepodudaranje: A) DWI hiperintezni signal u predelu levog hemiaspekta ponsa; B) Mapa vrednosti difuzionog koeficijenta sa odgovarajućom hipointenznom pontinskom zonom; C) FLAIR sekvenca bez izmene signala u predelu ponsa*

strongly indicative of acute blood vessel thrombosis. Computed tomography angiography (CTA) confirmed BA occlusion **(Figure 1b)**. Magnetic resonance imaging (MRI) was performed next, showing an area of restricted diffusion on DWI on the left side of the pons **(Figure 2a)**, without FLAIR signal hyperintensity **(Figure 2c)**, confirming DWI-FLAIR mismatch. A MT was done, 16 hours after patient was seen well, using both aspiration and stent retriever, and modified treatment in cerebral ischemia score 3 was achieved. The NIHSS score after the procedure was 9, and after 24 hours it was 2, while the patient had only mild right hemiparesis. The patient was prescribed a direct oral anticoagulant for secondary prevention, and at discharge his NIHSS and modified Rankin scale were 0.

Discussion

In Vojvodina, MT was introduced as a standard operative procedure for acute stroke treatment due to large vessel occlusion in 2016 [5]. We present a patient with wake-up stroke due to BA occlusion, with DWI-FLAIR mismatch, treated with MT. Wake-up strokes are common in everyday practice, and can be met in up to 44% of all stroke cases [6]. The DWI-FLAIR mismatch can be used for identifying patients eligible for thrombolysis whose symptoms onset was within 4.5 hours, even if the time of onset is unknown [7, 8]. This mismatch has not been used in larger trials for patient assessment and MT.

Posterior circulation is rich in collaterals and anastomotic channels, as pons specifically receives blood supply from proximal and middle segments of BA branches [1]. In accordance with these statements, our patient probably had good collateral circulation status that kept him FLAIR-negative even after 15 hours (when MRI was done) from last seen well.

The DWI-FLAIR mismatch may be useful in the assessment of patients with posterior circulation strokes with unknown-onset because the affected territory is smaller than in anterior circulation, so MRI must always be used due to its higher resolution, sensitivity, and specificity for ischemic events than CT. In addition, there are no optimized diagnostic tools to detect the penumbra zone with perfusion modalities because the brainstem, cerebellum and posterior cerebral artery territory receives a small blood supply volume [9]. This is why DEFUSE 3 and DAWN trial criteria are not fully applicable for brainstem infarctions. Also, patient selection without using perfusion and/or semi-automated software is reproducible and feasible in many centers [2].

Conclusion

We have shown that mechanical thrombectomy may be used for the treatment of basilar artery occlusion beyond 6 hours from the onset of symptoms and with diffusion-weighted imaging/fluid attenuated inversion recovery magnetic resonance imaging mismatch, but further research and randomized controlled trials are needed for this kind of treatment to be included in future recommendations.

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PROSTHETIC HEART VALVE THROMBOSIS – A HEADACHE FOR THE HEART TEAM – A CASE REPORT

TROMBOZA VEŠTAČKOG SRČANOG ZALISKA – GLAVOBOLJA ZA TIM ZA SRCE – PRIKAZ SLUČAJA

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Summary

Introduction. Prosthetic valve thrombosis is usually a subacute or chronic condition, although it may also present with a fresh thrombus. It occurs in two forms: obstructive and non-obstructive thrombosis. **Case Report.** We present a case of a female patient who underwent mitral valve replacement with mechanical prosthesis due to severe mitral stenosis. The postoperative course was uneventful and the patient was discharged on vitamin K antagonist therapy with international normalized ratio target 3.0. Five months later, the patient was admitted with severe shortness of breath and signs of acute heart failure. International normalized ratio at that moment was 2.3. Transthoracic echocardiography indicated severely raised gradient across the prosthetic valve and mechanical valve malfunction was suspected. Cinefluoroscopy showed that one of the prosthetic valve leaflets was completely immobile. Transesophageal echocardiography definitely confirmed thrombosis of the prosthetic valve with large multiple thrombi that completely fixed one leaflet in closed position, and partially limited the motion amplitude of the other leaflet. There were thrombi floating between the left ventricle and left atrium. Thrombectomy of the prosthetic valve was performed, which was sufficient for the complete restoration of the mechanical valve function. The vitamin K antagonist dosage was carefully up-titrated in order to reach and maintain the target international normalized ratio of 3.0. **Conclusion.** Prosthetic valve thrombosis is a serious and life-threatening condition that requires urgent management. Coordination and cooperation of the whole heart team is necessary for optimal choice of treatment, which primarily includes surgery or fibrinolysis.

Key words: Heart Valve Prosthesis; Thrombosis; Mitral Valve; Heart Valve Prosthesis Implantation; Anticoagulants; Vitamin K; Signs and Symptoms; Diagnosis; Reoperation

Sažetak

Uvod. Tromboza veštačkog zaliska obično predstavlja subakutno ili hronično stanje, mada može biti i akutno sa svežim trombom. Tromboza može da se javi se u dve forme - kao opstruktivna i neopstruktivna. **Prikaz slučaja.** Prikazujemo slučaj pacijentkinje kojoj je zamenjen mitralni zalistak mehaničkom protezom zbog teške stenoze. Postoperativni tok je protekao uredno i pacijentkinja je otpuštena sa terapijom antagonistom vitamina K sa ciljnim internacionalnim normalizovanim odnosom od 3,0. Pet meseci kasnije, pacijentkinja je primljena zbog gušenja i znakova akutne srčane slabosti. Vrednost protrombinskog vremena iznosila je 2,3. Transtorakalna ehokardiografija ukazala je na značajno povišene gradijente nad veštačkim zaliskom i postavljena je sumnja na malfunkciju mehaničke valvule. Sinevalvulografija je pokazala da je jedan listić veštačkog zaliska potpuno nepokretan. Transezofagealna ehokardiografija je definitivno potvrdila trombozu veštačkog zaliska sa multiplim velikim trombima koji su u potpunosti fiksirali jedan listić u zatvorenoj poziciji, a takođe delimično ograničili amplitudu pokreta drugog listića. Trombne mase su flotirale između leve komore i leve pretkomore. Načinjena je trombektomija veštačkog zaliska, što je bilo dovoljno za potpuni oporavak funkcije mehaničkog zaliska. Doza antagoniste vitamina K je pažljivo povećana kako bi se dostigla i održala ciljna vrednost protrombinskog vremena od 3,0. **Zaključak.** Tromboza veštačkog zaliska je ozbiljno i životnougrožavajuće stanje koje zahteva hitno zbrinjavanje. Za optimalan izbor lečenja, koji primarno obuhvata operaciju ili fibrinolizu, neophodna je koordinacija i saradnja celog tima za srce.

Ključne reči: veštački srčani zalistak; tromboza; mitralni zalistak; implantacija veštačkog zaliska; antikoagulanti; vitamin K; znaci i simptomi; dijagnoza; reoperacija

Introduction

More than 100 million people worldwide are affected by valvular heart disease and surgical aortic valve replacement is the treatment of choice (or repair of mitral valves). There are two different types of surgical prosthetic heart valves: mechanical heart valves and bioprosthetic heart valves. All implanted prosthetic valves are thrombogenic, especially mechanical, yet they are more durable than biological [1].

The pathogenesis of intracardiac thrombus formation is complex. According to Virchow's triad, the predisposing factors to thrombus formation can be divided into endothelial, hemodynamic, and hemostatic factors. Endothelial or surface factors include biocompatibility of the prosthesis and interactions between the prosthesis and the suture zone. Hemodynamic factors include the patient's cardiac hemodynamic status, as well as the intrinsic hemodynamic characteristics of the prosthetic valve. Last but not least, there are he-

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Abbreviations

NYHA – New York Heart Association
TTE – transthoracic echocardiography
MVA – mitral valve area
BSA – body surface area
VKA – vitamin K antagonist
INR – international normalized ratio
TEE – transesophageal echocardiography
CT – computed tomography

mostatic factors that involve the adequacy of anticoagulant treatment [1, 2]. The risk of thrombus formation, despite adequate oral anticoagulation, is estimated to be between 1 - 4% per year [3].

Prosthetic valve thrombosis is usually a subacute or chronic condition, although it may also present with a fresh thrombus [2]. It occurs in two forms: obstructive and non-obstructive thrombosis. The incidence of obstructive prosthetic valve thrombosis varies between 0.3 - 1.3% patient-years. On the contrary, the non-obstructive form is a relatively frequent finding in the postoperative period (as high as 10%), although thromboembolic complications are more frequent in the obstructive form, and occur at a rate of 0.7 - 6% patient-years [2].

Prosthetic valves in the tricuspid position have the greatest thrombogenic potential (up to 20%), followed by the mitral position (0.5 - 8%) [4]. According to a series of surgical interventions for prosthetic valve thrombosis, the first postoperative year is marked by 24% incidence of thrombosis, with a stable incidence between the second to fourth years of approximately 15%, with a subsequent decrease thereafter [2].

Case Report

A 60-year-old female patient was admitted for surgical treatment of mitral valve disease. She complained of fatigue, dyspnea on minimal exertion and occasional palpitations, while her New York Heart Association (NYHA) class was III. The symptoms started one year before and gradually increased over time. Her only comorbidity was hypothyroidism that was well controlled with substitution therapy. Physical examination showed a diastolic murmur of low-frequency over the mitral valve.

Transthoracic echocardiography (TTE) showed severe mitral stenosis with thickened, fibrous degenerative mitral cusps and mitral annular calcification **(Figure 1A)**. Maximal and mean transmitral gradients were 24 and 13 mmHg, respectively **(Figure 1B)**. Planimetry showed mitral valve area (MVA) of 0.8 cm^2 (0.4 cm^2/m^2 indexed for body surface area (BSA)). Mitral annulus was dilated at 3.9 cm and moderate mitral regurgitation was also registered. Left atrium was severely dilated with 5.9 cm in diameter and 182 ml volume (90.5 ml/m^2 indexed for BSA). Moderate tricuspid regurgitation was present as well. The systolic function of both ventricles was preserved (left ventricular ejection fraction 60%; tricuspid annular plane systolic excursion 22 mm).

Preoperative coronary angiography was without lesions, so coronary artery disease was excluded, and

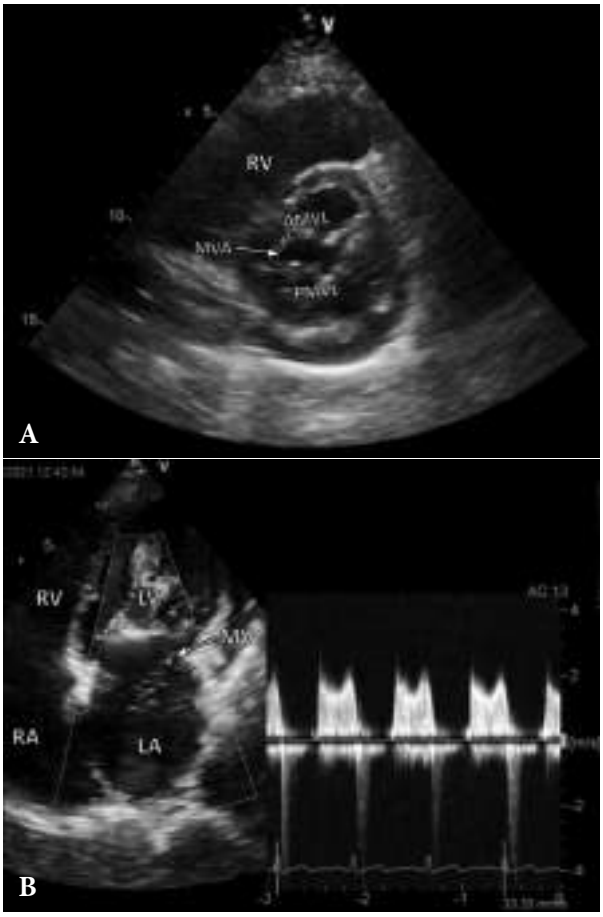

Figure 1. Transthoracic echocardiography on admission: A) mitral valve stenosis from parasternal short-axis view; B) transmitral flow with continuous wave Doppler, maximal gradient 24 mmHg, mean gradient 13 mmHg

Slika 1. *Transtorakalna ehokardiografija pri prijemu: A) stenoza mitralnog zaliska na parasternalnom poprečnom preseku; B) transmitralni protok kontinuiranim doplerom, maksimalni gradijent 24 mmHg, srednji gradijent 13 mmHg*

Legend: AMVL – anterior mitral valve leaflet; LA – left atrium; LV – left ventricle; MV – mitral valve; MVA – mitral valve area; PMVL – posterior mitral valve leaflet; RA – right atrium; RV – right ventricle

Legenda: AMVL – prednji mitralni kuspis; LA – leva pretkomora; LV – leva komora; MV – mitralni zalistak; MVA – površina mitralnog zaliska; PMVL – zadnji mitralni kuspis; RA – desna pretkomora; RV – desna komora

heart valve surgery was indicated by the heart team. The patient underwent surgery in general endotracheal anesthesia with extracorporeal circulation. Mitral valve replacement with a mechanical prosthesis (On-X No. 27/29) with preservation of P2 and P3 scallops, along with tricuspid valve repair were performed. Histopathological analysis of the removed mitral valve tissue showed fibromyxomatous degeneration of mitral leaflets. The postoperative course was uneventful and the patient was discharged on the 8th postoperative day in good general condition on vitamin K antagonist (VKA) therapy with international normalized ratio (INR) target 3.0.

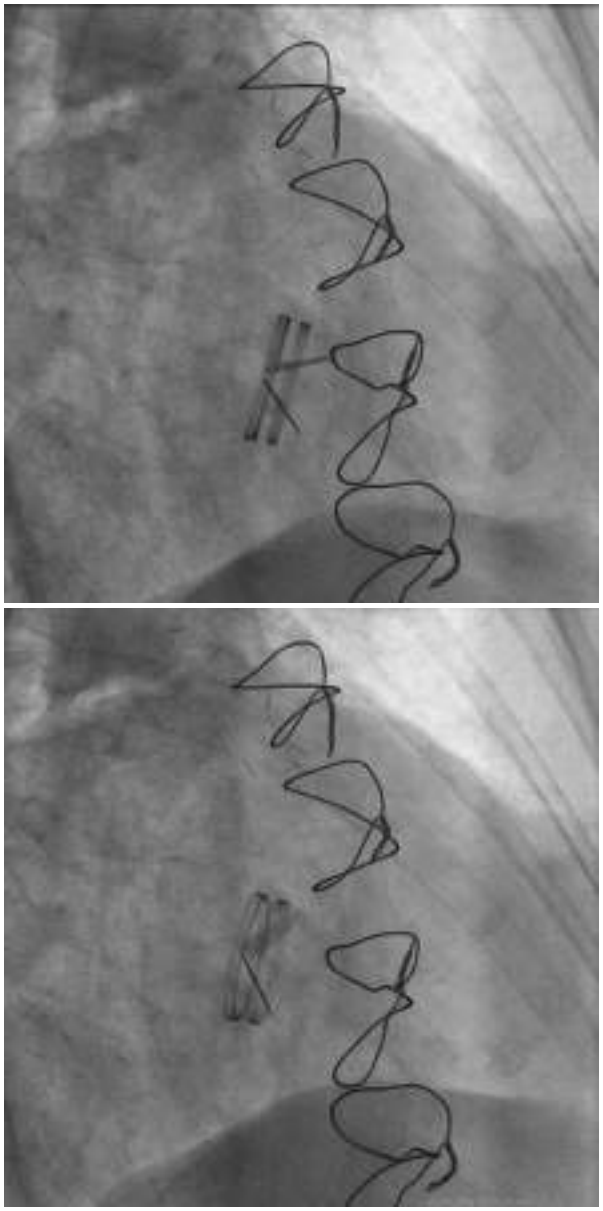

Figure 2. Cinefluoroscopy of the prosthetic valve shows complete immobility of one leaflet
Slika 2. *Sinefluoroskopija veštačke valvule: jedan listić je u potpunosti nepokretan*

One month later, the patient was admitted as an emergency due to dyspnea and palpitations. Echocardiography showed cardiac tamponade with large pericardial effusion, so an emergency subxiphoid pericardial drainage was performed and 500 ml of sero-hemorrhagic effusion was evacuated. The chest tubes were removed two days later so VKA was discontinued and bridge therapy with low-molecular weight heparin was administered. After chest tube removal, VKA was continued and the patient was discharged.

Five months after the heart valve surgery and mechanical valve implantation, the patient presented with severe shortness of breath and signs of acute heart failure, with NYHA class IV. A TTE was immediately performed and revealed severely elevated maximal and mean pressure gradients across prosthetic valve (43 and 24 mmHg, respectively) and mechanical valve malfunction was suspected. The INR value at that moment was 2.3, N-terminal pro-brain natriuretic peptide was elevated (13.583 pg/ml), while other blood laboratory test results were normal. Immediately after admission, continuous intravenous infusion of unfractionated heparin was initiated.

Cinefluoroscopy showed that one of the prosthetic valve leaflets was completely immobile, while the other was moving properly **(Figure 2)**. Transesophageal echocardiography (TEE) was also performed for confirmation. Thrombosis of the prosthetic valve was confirmed, with large multiple thrombi that have completely fixed one leaflet in closed position, and also partially limited the motion amplitude of the other leaflet. There were thrombi floating between the left ventricle and left atrium **(Figure 3)**.

After a detailed heart team evaluation, urgent surgical reoperation was indicated. Following a 72-hour clinical stabilization, the patient once again underwent

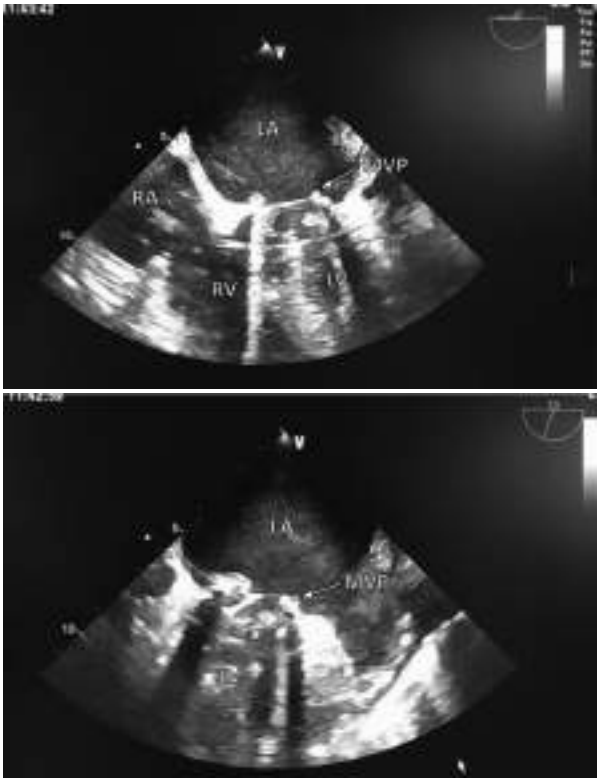

Figure 3. Transesophageal echocardiography of the prosthetic valve: thrombosis with multiple thrombi floating between the left ventricle and left atrium
Slika 3. *Transezofagealna ehokardiografija veštačke valvule: tromboza sa multiplim trombima koji flotiraju između leve komore i leve pretkomore*
Legend: LA – left atrium; LV – left ventricle; MVP – mitral valve prosthesis; RA – right atrium; RV – right ventricle
Legenda: LA – leva pretkomora; LV – leva komora; MVP – veštački mitralni zalistak; RA – desna pretkomora; RV – desna komora

surgery in general endotracheal anesthesia with extracorporeal circulation. Thrombectomy of the prosthetic valve was performed, which was sufficient for complete restoration of the mechanical valve function, as confirmed by perioperative TEE. The postoperative course was uneventful, the patient was stable and without any symptoms, so she was discharged on the 8th postoperative day in good general condition. Her VKA dosage was carefully up-titrated in order to reach and retain the target INR of 3.0. Subsequent control TTE showed normal function of the mechanical prosthesis, with normal pressure gradients across the valve.

Discussion

Prosthetic valve thrombosis is one of the most dangerous complications following surgical valve replacement and it is associated with high morbidity and mortality. Dysfunction of the prosthetic valve, with or without thrombosis, may clinically manifest as progressive dyspnea and signs of heart failure [5]. Prosthetic valve thrombosis may be detected incidentally during routine echocardiography examination. Also, in every patient with acute or subacute development of symptoms and progression of transprosthetic gradient (compared to previous echocardiography examination) there is a suspicion of a possible prosthetic valve dysfunction [6–8]. This case report presents a way to detect and solve a complex problem called mechanical valve thrombosis.

The intensity and duration of anticoagulant therapy depend on the type of prosthesis, risk of bleeding, and thromboembolic complications [9–12]. Patients with implanted mechanical valve require lifelong anticoagulant therapy with VKA [13]. Lin et al. described several predictors of mechanical valve thrombosis and they include occlusive mobile mass, increased transvalvular pressure, and INR ≤ 2.5 [14]. In the German Experience with Low Intensity Anticoagulation study, patients with mechanical valve at the mitral position and low INR were associated with lower survival rates compared to patients with higher INR values of 2.5 - 4.5 [15]. According to the latest recommendations, in patients with mechanical valve at mitral position, anticoagulant therapy with VKA is indicated to achieve the INR target value of 3.0 [16]. One of the possible explanations for the thrombosis of the mechanical valve in our patient may be inadequate anticoagulant therapy and subtherapeutic INR value of 2.3 on admission, which can be affected by a number of factors such as age, weight, diet and interaction with other drugs [17].

The TTE is the first line test for the diagnosis of prosthetic valve dysfunction [18]. Although this method has many advantages, such as assessment of hemodynamics and valve motility, TTE is limited in morphological characterization of the etiology of mechanical valve dysfunction. Acoustic shadowing caused by the prosthesis itself can make visualizing blood clots, pannus, and vegetation more difficult, but other factors can also affect the accuracy of this method, such as the presence of pericardial effusion, previous sternotomy, and obesity [18]. Due to the above mentioned, for better evaluation of prosthetic valve dysfunction after TTE, TEE should be performed, which has a significant role in guiding the therapeutic strategy. Furthermore, in left-sided prosthetic valve thrombosis, the presence of thrombus favors surgery because fibrinolysis carries a significant risk of embolism. The mobility of the mechanical valve can also be assessed by cinefluoroscopy, which enables the assessment of the opening angles of the prosthesis [16]. In a number of cases, multidetector computed tomography (CT) can provide an accurate assessment of prosthetic valve structure and functional status [19, 20]. According to the latest recommendations for the treatment of valvular heart diseases, every patient with suspected mechanical valve thrombosis needs an urgent evaluation by TTE, TEE and/or multidetector CT [16, 21]. Following the latest recommendations, our patient underwent TTE, TEE and cinefluoroscopy, which altogether gave insight into the correct diagnosis and enabled an adequate treatment strategy.

There are several therapeutic options for mechanical valve thrombosis, including emergency surgery, thrombolytic therapy, and anticoagulation therapy. According to the 2020 American College of Cardiology/American Heart Association recommendations [16], the decision on either emergency surgery or systemic fibrinolysis should be made by the heart team based on the individual characteristics of each patient. Emergency surgery is recommended for patients with NYHA functional class III or IV and/or large thrombus (thrombus area ≥ 0.8 cm^2), while NYHA classes I and II, high operative risk, low risk of bleeding, and a small thrombus favor fibrinolysis as a therapeutic option. In contrast, according to the 2021 European Society of Cardiology and the European Association for Cardio-Thoracic Surgery guidelines [21], surgery is preferred and fibrinolysis should only be used when surgery is not an option. Due to the presence of a large thrombus, acute heart failure, absence of significant comorbidities and low operative risk in our patient, emergency surgery was indicated as a therapeutic option. In their meta-analysis, Karthikeyan et al. included several studies comparing surgery and fibrinolysis as therapeutic options for mechanical valve thrombosis [22]. They concluded that emergency surgery led to complete success in 86.5% of cases and fibrinolysis in 69.7%. Significant bleeding was less common in operated patients (1.4% vs. 5%). Stroke was less common in the group of operated patients compared to patients undergoing fibrinolysis, and also repeated mechanical valve thrombosis occurred less frequently in operated patients.

Conclusion

Prosthetic valve thrombosis is a serious life-threatening condition that requires urgent management. Multimodality imaging is crucial for evaluating the artificial valve function and establishing the correct diagnosis. Coordination and cooperation of the whole heart team is necessary for optimal choice of treatment, which primarily includes either surgery or fibrinolysis.

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CORONAVIRUS - CLINICAL MANIFESTATIONS BY ORGAN SYSTEMS AND PROGNOSIS

KORONAVIRUS – KLINIČKE MANIFESTACIJE PO ORGANSKIM SISTEMIMA I PROGNOZA

Milica GOJKOVIĆ[1], Milanka TATIĆ[2, 3] and Sanja MARIČIĆ PRIJIĆ[3, 4]

Summary

Introduction. Coronaviruses were first isolated in 1962, and they belong to the family Coronaviridae, subfamily Orthocoronavirinae. Coronavirus disease 2019 is a complex multisystem disease that affects all organ systems. **Respiratory Manifestations**. Coronavirus disease 2019 pneumonia may present with fever, weakness, malaise, tachypnea and dyspnea. Acute respiratory distress syndrome is the most severe form of lung damage, manifested by pronounced respiratory insufficiency that requires oxygen therapy. **Cardiovascular Disorders**. The most common clinical presentations of coronavirus disease 2019 infection are: acute coronary syndrome - myocardial infarction, arrhythmias, myocarditis, myopericarditis, pericardial effusion, and thromboembolic complications. **Neurological Disorders**. The most common neurological symptom is headache, which is caused by activation of the trigeminal nerve endings due to the coronavirus infection. The severe forms of the disease are associated with ageusia, anosmia, and disorders of consciousness, concentration problems, and encephalopathy. **Renal Disorders**. The coronavirus infection affects all kidney structures with preferential tropism for glomerular cells. There are two types of kidney damage; mild and severe form requiring hemodialysis. **Skin Changes**. Skin changes appear due to coagulation disorders and inflammation of the walls of small blood vessels. **Ophthalmic Manifestations**. The most important ophthalmic manifestation is follicular conjunctivitis, which may appear in the early and late stages of the disease. **Conclusion**. The prognosis of coronavirus disease 2019 depends on the involvement of organ systems, the age of the patient, associated comorbidities, and the time of treatment initiation.

Key words: COVID-19; Coronavirus Infections; Diagnosis; Signs and Symptoms; Prognosis; Risk Factors; Severe Acute Respiratory Syndrome; Cardiovascular Diseases

Sažetak

Uvod. Koronavirusi su prvi put izolovani 1962. godine, pripadaju porodici *Coronavirinae* potfamiliji *Orthocoronavirinae*. Koronavirus je kompleksna multisistemska bolest koja ostavlja posledice na sve organske sisteme. **Respiratorne menifestacije.** U sklopu COVID pneumonije prisutni su: povišena temperatura, slabost, malaksalost, anosmija, ageuzija, tahipnea i dispnea. Akutni respiratorni distres sindrom predstavlja najteži oblik oštećenja pluća, manifestuje se izraženom respiratornom insuficijencijom koja zahteva visoku potporu kiseonikom. **Kardiovaskularni poremećaji.** Najčešće kliničke prezentacije koronavirusne infekcije su: akutni koronarni sindrom − infarkt miokarda, aritmije, miokarditis, mioperikarditis, perikardna efuzija i tromboembolijske komplikacije. **Neurološki poremećaji.** Najčešći neurološki simptom je glavobolja koja nastaje aktivacijom završetaka trigeminalnog nerva usled infekcije koronavirusom. Kod težih formi bolesti prisutni su: ageuzije, anosmija, poremećaj svesti, koncentracija i encefalopatija. **Bubrežni poremećaji.** Uticaj koronavirusne infekcije registrovan je u svim bubrežnim strukturama sa predominantnim tropizmom prema glomerulskim ćelijama. Postoje dva tipa bubrežnog oštećenja − blaga i teška forma koja zahteva hemodijalizu. **Promene na koži.** Kožne promene se pojavljuju zbog poremećaja koagulacije i inflamacije zida malih krvnih sudova. **Oftalmološke manifestacije.** Najznačajnija oftalmološka manifestacija je folikularni konjuktivitis koji se može pojaviti u ranoj i kasnoj fazi bolesti. **Zaključak.** Prognoza bolesti zavisi od reperkusija na organske sisteme koronavirusne infekcije, starosti bolesnika, pridruženih komorbiditeta i momenta započinjanja lečenja.

Ključne reči: COVID-19; infekcije korona virusom; dijagnoza; znaci i simptomi; prognoza; faktori rizika; akutni respiratorni sindrom; kardiovaskularna oboljenja

Introduction

Coronaviruses were first isolated in 1962, and have been shown to cause disease in mammals and birds [1]. Under the electron microscope, they have the shape of a crown due to the presence of glycoproteins on the viral surface. They are positive-strand ribonucleic acid chains which belong to the family of Coronaviridae, subfamily Orthocoronavirinae. There are 7 types of coronaviruses that infect humans [2].

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Abbreviations

SARS-CoV-2	– severe acute respiratory syndrome coronavirus 2
COVID-19	– coronavirus disease 2019
ARDS	– acute respiratory distress syndrome
ACE-2	– angiotensin-converting enzyme
PaO_2	– partial pressure of oxygen in arterial blood
FiO_2	– fraction of inspired oxygen concentration
PEEP	– positive end-expiratory pressure
TLM	– Time/Lymphocyte Percentage Model
LYM	– Lymphocyte%

In December 2019, in the province of Wuhan in the city of Hubei, China, cases of pneumonia of unknown etiology appeared, and on January 7, it was confirmed that severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) was the cause of the disease [3]. It is a new strain of the virus that has been identified for the first time in the human population. In genetic structure, it is similar to SARS-CoV [4].

Coronavirus disease 2019 (COVID-19) is a global health problem. It is a complex multisystem disease. The SARS-CoV-2 attacks host cells by binding to its spike glycoprotein (S) metallopeptidase called angiotensin-converting enzyme (ACE-2). The ACE-2 is present in various tissues of the body, namely in the oral and nasal mucosa, nasopharynx, lungs, kidneys, brain, heart, liver, spleen, stomach, small intestine, colon, lymph nodes, thymus, and bone marrow [5]. These receptors are present in alveolar epithelial cells of the lungs, as well as endothelial muscle cells of arteries and veins. The immune response of the infected host is intense, leading to the hyperinflammatory syndrome "cytokine storm", which is characterized by an increase in proinflammatory cytokines such as tumor necrosis factor alpha, interleukin-6 and chemokine [5, 6]. The infection manifests itself in various forms: from mild asymptomatic to severe life threatening, accompanied by respiratory failure, sepsis and multiorgan dysfunction. Risk factors for the development of a severe form of the disease are: age, associated cardiovascular, pulmonary, neurological, renal comorbidities, malignancies and various immunodeficiency states. This disease affects all organ systems.

Respiratory Manifestations

The most common symptoms of this disease include fever, weakness, malaise, anosmia, ageusia, cough, tachypnea and dyspnea [7]. These symptoms are present in coronavirus pneumonia. It is a viral interstitial pneumonia that can be further complicated as a bacterial superinfection.

Acute respiratory distress syndrome (ARDS) is the most severe form of lung damage caused by SARS-CoV-2 infection, manifested by severe respiratory failure requiring oxygen therapy [8]. It is an acute, diffuse, inflammatory damage to the lungs that leads to increased vascular permeability, increased lung weight and loss of the ventilatory part of the lung parenchyma **(Figure 1)**. The clinical picture includes hypoxemia, radiographic bilateral pulmonary patchy shadows, mixing of arterial and venous blood (shunting), increase in dead space, and decrease in pulmonary compliance. It is characterized by diffuse alveolar damage that occurs due to damage to the endothelium of alveolar capillaries and due to damage to the alveolar epithelium [8, 9].

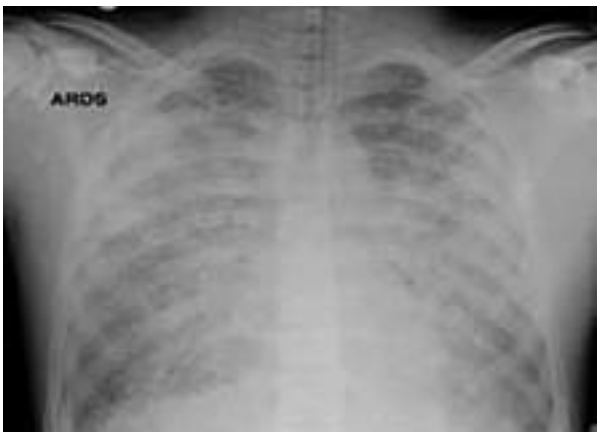

Figure 1. Acute respiratory distress syndrome - chest X-ray
Slika 1. *Akutni respiratorni distress sindrom – radiografski prikaz*

The COVID-19-related ARDS is divided into three categories:

1. Mild ARDS 200 mmHg < (ratio of partial pressure of oxygen in arterial blood - PaO_2 to the fraction of inspired oxygen concentration - FiO_2) PaO_2/FiO_2 < 300 mmHg (for positive end-expiratory pressure - PEEP > 5 cmH_2O);
2. Moderate ARDS 100 mmHg < PaO_2/FiO_2 < 200 mHg (for PEEP > 5 cm H_2O);
3. Severe ARDS PaO_2/FiO_2 < 100 mmHg (for PEEP > 5 cmH_2O).

Neutrophils and platelets that accumulate in the pulmonary capillaries play a major role in endothelial damage. Reduced pulmonary blood flow allows these cells to adhere to endothelial cells and start acting on each other [9]. Various particles are released from damaged endothelial cells, and the blood coagulation system, quinine and other inflammatory factors are activated. Complement activation attracts other inflammatory cells, so the process of alveolar-capillary membrane damage continues in autocatalytic manner and can hardly be interrupted [10, 11].

Damage to alveolar cells occurs due to inflammatory mediators, which leads to increased permeability of the alveolar-capillary membrane; transudation of plasma into alveoli causes non-cardiogenic pulmonary edema [11]. Fibrin polymerization occurs on the surface of alveoli and turns into microscopically visible deposits, the so-called hyaline membranes. The COVID-19 damages type I and type II pneumocytes. Damage to type I pneumocytes causes a decrease in respiratory area-hypoxia, while damage to type II pneumocytes reduces the production of surfactant with consequent atelectasis. These changes reduce respiratory lung capacity. Diffuse alveolar damage is divided into three phases: exudative, proliferative, and fibrous phase [11].

The exudative phase lasts several days and is characterized by airless lungs, with interstitial and alveolar edema, after which formation of hyaline membranes that line the surface of the alveolar septa occurs. The exudative phase is accompanied by a high mortality rate.

The proliferative phase lasts from one to three weeks and granulocyte tissue is present in the lung parenchyma. The fibrous phase is present between the third and fourth week. Lung tissue is diffusely fibrous.

Clinically, ARDS is manifested by dyspnea and refractory hypoxemia; the mortality rate is high, between 30 - 40%. Patients with associated comorbidities have a worse prognosis [12].

Cardiovascular Disorders

During COVID-19 infection, direct and indirect cardiovascular disorders are possible. Direct infection of cardiomyocytes, pericytes and fibroblasts induces myocardial damage with cardiotoxic effects. Due to hypoxia, microvascular damage of coronary blood vessels, endothelial cell infection and diffuse inflammation occur. The most common clinical presentations of COVID-19 infection are: acute coronary syndrome (myocardial infarction), arrhythmias (supraventricular arrhythmias, ventricular tachycardia and fibrillation, impulse conduction disorders), heart failure accompanied by systolic dysfunction, myocarditis, myopericarditis, pericardial effusion, and thromboembolic complications [12, 13]. Cardiovascular consequences occur most often in the advanced phase of the disease, in patients with a greater need for oxygen support and the progression of radiological findings. Early recognition is important in order to positively influence the outcome of the disease [13].

Coronavirus affects hypercoagulability with a prevalence of 25 - 43%. Elevated levels of fibrinogen and D-dimer are laboratory indicators of coagulation disorders, with discretely prolonged prothrombin time and partial thromboplastin time, while platelet count is slightly elevated or decreased [13]. These coagulation disorders occur in the complex interaction of vascular endothelial damage directly caused by infection or indirectly by inflammatory mediators. Hypercoagulability is responsible for the expression of tissue factor under the influence of activated mononuclear cells and interleukin-6 as well as direct viral infection of endothelial cells with the release of plasminogen activators and von Willebrand factor multimers [14]. A study conducted in Amsterdam proved that the usual prophylaxis of deep vein thrombosis did not affect the formation of micro thrombi during the COVID-19 infection [14, 15].

Neurological Disorders

Coronaviruses may have a neuroinvasive potential that, in addition to anosmia caused by olfactory bulb damage, ageusia, feelings of instability and disorders in concentration, can cause impaired consciousness (epileptic attack and delirium) in severe forms of the disease. Encephalopathy, which is present in the severely ill, is a consequence of the action of cytokines [16, 17].

The most common symptom is headache caused by activation of trigeminal nerve endings due to direct infection with coronavirus or indirectly through circulating proinflammatory cytokines, due to hypoxia and vasculopathy. The headache is usually bilateral, localized frontally, temporo-parieto-occipital or periorbital, pulsating in character, with poor response to analgesics [17].

Renal Dysfunction

The COVID-19 infection affects all kidney structures with preferential tropism for glomerular cells. Mild to severe renal impairment requires hemodialysis [17]. Renal damage with respiratory deterioration accompanied by radiological progression and increased need for oxygen support is a predictor of lethal outcome. In a Spanish study of 36 patients with renal insufficiency requiring hemodialysis, 30.5% were fatal [18].

Skin Changes

The most common skin changes appear in the distal parts of the toes in the form of erythematous or livid macules, plaques and nodules. They occur due to coagulation disorders and inflammation of the small blood vessel walls. These changes are not indicators of the severity of the disease, they most often occur in mild forms [17]. There are also changes in the form of vesicles and pustules on an erythematous base. Vesicles appear in the early phase of the disease before the onset of symptoms or around the third day and they are usually localized on the trunk. Painful erosions and ulcerations, erythematous changes and aphthous ulcers appear on the mucosa, with the possible appearance of extensive changes in the form of gingivitis [18] **(Figure 2).**

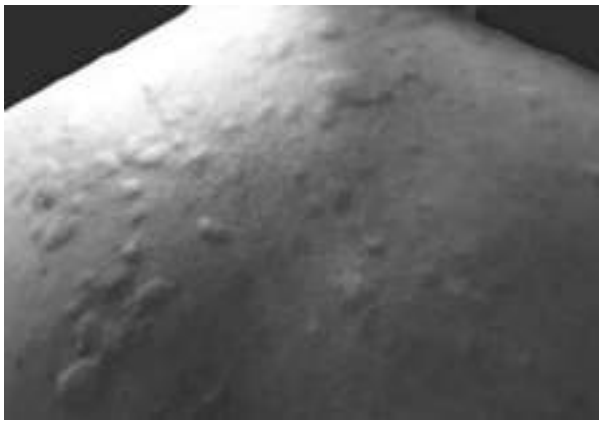

Figure 2. Skin changes in COVID-19 infection
Slika 2. *Promene na koži u COVID-19 – infekciji*

Ophthalmic Manifestations

The most important ophthalmic manifestation in COVID-19 infection is follicular conjunctivitis, which may occur in the early and late stages of the disease. In most cases, it is bilateral, accompanied by redness, conjunctival hyperemia, chemosis, epiphora and in-

Figure 3. Conjunctivitis in COVID-19 infection
Slika 3. *Konjuktivitis u COVID infekciji*

crease in tear secretion. In some patients, there are changes in the retinal ganglion cells which cause permanent visual impairment [19] **(Figure 3)**.

Prognosis

In severe forms of the disease, the prognosis is poor, accompanied by a high mortality rate up to 60% [20].

Unfavorable prognosis indicators:

- Age ≥ 65, men.
- Associated diseases: heart disease (including arterial hypertension), lung disease, diabetes, malignant disease, immunosuppression.
- Laboratory findings: severe lymphopenia, elevated troponin, urea, creatinine, lactate dehydrogenase, C-reactive protein, and D-dimer.

In Italy, the Time/Lymphocyte Percentage Model (TLM) is also used, i.e. lymphopenia is an effective and valuable indicator for determining the severity of the disease in hospitalized patients with COVID-19. The TLM is based on the fact that patients have different percentages of lymphocytes after the onset of COVID-19 infection [20].

The TLM-1 (Time Lymphocyte% (LYM) Model), 10 to 12 days after the onset of symptoms, patients with LYM > 20% are initially classified as 'moderately severe' and are cured relatively quickly. Patients with LYM < 20% should initially be classified as 'severe cases' [21].

The TLM-2 (Time Lymphocyte% Model), approximately 17 - 19 days after the onset of symptoms, patients with LYM > 20% are recovering, 5% < LYM < 20% are still at risk and need attention, LYM < 5% are critical cases, with a high mortality rate and the need for treatment in intensive care units [22].

Lethal outcomes caused by COVID-19 infection

- 53% respiratory failure
- 33% concomitant respiratory and heart failur
- 7% heart failure.

The recovery depends on the age, comorbidities and the severity of the clinical picture. Recovery from a mild infection takes up to two weeks, while more severe forms of the disease take three to six weeks. Symptoms such as fatigue, coughing, and difficulty returning to daily activities may persist for a long period of time after illness. Recovery of lung and respiratory function in patients with a mild form of the disease occurs within six months of the onset of the first symptoms. In patients with a more severe form of the disease who require hospitalization but not admission to intensive care unit, recovery is established within 12 months [23]. In patients with the most severe form of the disease, who require mechanical ventilation during hospitalization, pulmonary manifestations persist in the form of restrictive lung disease, decreased pulmonary diffusion capacity and fibrosis, which directly affects the quality of life [24].

Conclusion

The prognosis of coronavirus disease 2019 depends on the involvement of organ systems, the patient's age, associated comorbidities, and the time of initiation of therapy. It is very important to know the clinical manifestations of this disease well, because timely recognition and treatment reduce the possibility of complications and more severe forms of the disease. The recovery is individual, depending on the age, comorbidities and severity of the clinical picture.

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4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

6. Prikazi slučajeva – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings, MeSH.*

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.