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Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
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The building in Belgrade, tavern "?" – "Question mark", which exists to this day, in which Matija Ivanović opened a private pharmacy in 1830. See more about the first pharmaceutical institutions in Serbia in the paper by Aleksandar Nedok "The oldest apothecaries of the Serbian Armed Forces" (pp. 1054–1065).

Zgrada u Beogradu, kafana „?" – „Znak pitanja", koja i danas postoji, u kojoj je 1830. godine Matija Ivanović otvorio privatnu apoteku. Više o prvim apotekarskim ustanovama u Srbiji videti u radu Aleksandra Nedoka „Najstariji apotekari srpske vojske" (str. 1054–1065).

Dear Authors, Editors, Peer Reviewers, and Readers of the *Vojnosanitetski pregled*,

we thank you for your cooperation and support in 2023 and wish you all the best in the upcoming 2024!

Merry Christmas and Happy New Year!

Editorial staff of the *Vojnosanitetski pregled*

Dragi autori, urednici, recenzenti i čitaoci *Vojnosanitetskog pregleda*,

uz zahvalnost na saradnji i podršci u 2023, želimo vam sve najbolje u nastupajućoj 2024. godini!

Srećna Nova godina i Božićni praznici!

Redakcija *Vojnosanitetskog pregleda*

LETTER TO THE EDITOR

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Response to "Contribution of Dr. Laza Popović to the development of Serbian and Yugoslav Sokol movement"

Odgovor na „Doprinos dr Laze Popovića razvoju srpskog i jugoslovenskog sokolskog pokreta"

Dear editors,

With great interest, we read an article in your esteemed journal about Laza Popović [1], one of the doyens of Croatian Radiology. As Popović founded the Central Roentgenology Institute at the School of Medicine, University of Zagreb, his picture still hangs on the wall in our central lecture hall at the University Department of Diagnostic and Interventional Radiology, University Hospital Center "Zagreb".

Related to the article, we would like to point out an incorrect fact, which we hope appeared only as an unintentional erratic fact, without any ideological forethought. It is stated in the paper that "As an outstanding Serb, a Yugoslav, and a member of Sokol, he had to flee from Zagreb to Belgrade in 1941". This statement is historically inaccurate as Professor Popović was elected in 1939 for the Senate of the still-existing Kingdom of Yugoslavia and moved to Belgrade in the summer of 1940 [2, 3]. As additional evidence for our statement, we suggest reading the article in Belgrade's newspaper "Politika" from June 14, 1940. In that article, Dr. Milorad Dragić writes about planned public health action of lung X-ray screening for all school children in Belgrade. He starts the article with the following sentence: "He now came to Belgrade and became our permanent fellow citizen. Dr. Laza Popović, a professor at the Zagreb University, is a famous, respectable, and popular national and cultural worker" [4]. A few months later, Professor Laza Popović published two articles in the same newspapers about his role in the turbulent November days of 1918 as he continued to live in Belgrade until 1945, where he died in, for us, unknown circumstances [5, 6].

Mislav Čavka *†, Stjepan Matković‡

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GENERAL REVIEW

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Psychologists as emergency first responders during a pandemic

Psiholozi kao hitni prvi odgovor tokom pandemije

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Key words:
counseling; covid-19; distance counseling; psychological first aid; psychology, social; resilience, psychological.

Ključne reči:
savetovanje; covid-19; savetovanje na daljinu; prva pomoć, psihološka; psihologija, socijalna; rezilijentnost, psihološka.

Introduction

While comparing the pandemic to World War II seems appropriate because of the magnitude of the impact it had around the world, there is one clear difference between the two most horrific world events of the last two centuries. During World War II, countries voluntarily chose to take sides – either participate with or against the Allied forces; the pandemic, on the other hand, was an involuntary, sudden crisis that was thrust upon all countries in the world. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rampaged through major political, economic, and social systems, destroying mental health and medical services. One of the most significant effects of the pandemic was the wrecking of the social fabric of societies and global communities [1]. The values of interdependence and social cohesion have been challenged by preventive measures like lockdowns, social distancing, and isolation undertaken by governments [2]. The core values and norms of human societies, such as the value attached to collective effervescence [3], the significance of social gatherings to mark important events, family gatherings, and membership in social groups, are threatened by common proactive measures such as quarantine and the need for social distancing. The pandemic has imposed a state of emergency characterized by immense uncertainty caused by multiple mutations of the SARS-CoV-2, ambiguity, and fear arising from the lack of knowledge on how to fully deal with the pandemic. The pandemic has brought about changes in all realms of social functioning. Although some countries and cultures have traditionally emphasized the role of collective resources and believe in the fair distribution of critical resources among the communities, the abrupt, explosive, and ambiguous nature of the pandemic resulted in the hoarding of resources by some and the exploitation of resources due to limited supply and access [4].

The literature clearly indicates that the reconstruction of a new world perspective in various spheres of life during and after the coronavirus disease 2019 (COVID-19) pandemic is an extremely complex phenomenon. That demands a multifaceted approach in order to better understand the theoretical underpinnings of the who, what, where, when, which, how, and why of the post-pandemic reconstruction, and more importantly, the application of psychological principles to the dynamic, yet fluid post-reconstruction process [5].

One of the reports from John Hopkins University of Medicine in 2020 indicated that by mid-August 2020, the SARS-CoV-2 had infected over twenty million people across the globe; this was about six months after it was declared a pandemic and caused over 750,000 deaths. Deaths due to infection, as well as separation from loved ones, may have resulted in a range of psychological problems [6] like anxiety due to the uncertain nature of the pandemic, feelings of helplessness [7, 8], cases of depression, feelings of loneliness and instances of suicide or suicide ideation [9], and feelings of anger and frustration due to high vulnerability to infection [10, 11]. Some of the subgroups, such as healthcare

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professionals, Emergency First Responders (EFRs), children, and the elderly population, are high-risk groups not only because of vulnerability to infection but also vulnerability to additional psychological costs of infection.

For instance, a study on paramedics revealed that paramedics who were in contact with patients with COVID-19 experienced higher stress compared to paramedics who were not in contact with patients. However, it was revealed that perseverance and determination, openness to new experiences, a sense of humor, as well as competencies and tolerance of negative emotions, played a key part in mitigating subjectively experienced stress [12].

Another study among nurses and midwives during the COVID-19 pandemic found that resilience plays a great role in shaping job satisfaction and intention to leave the job among medical professionals and that occupational stress weakens the relationship between resilience and intention to leave the job and between job satisfaction and resilience [13].

In some studies, it was found that children and adolescents are more vulnerable to developing anxiety symptoms and related disorders [7, 14]. Healthcare workers and EFRs are likely to develop symptoms typical of a stressful event such as a pandemic, including, amongst others, post-traumatic stress disorder (PTSD), emotional exhaustion, dissociation, burnout, prolonged social isolation due to stigmatization, etc. [15–17].

However, the pandemic-induced conditions, such as quarantine and social distancing, had some positive effects in terms of people becoming more sensitive in their interaction with others [18], having to spend more time at home has helped in improving relationships among family members [4], developing an empathetic approach to look at problems at large, to name a few. It has instilled in people several healthy habits like wearing masks, maintaining hygiene standards, social distancing, and the like. Necessity being the mother of invention, it is not a surprise that medical, technological, pharmaceutical, and scientific areas have benefited from new inventions and research acceleration during the pandemic. Most organizations had to adapt to new technologies, such as remote working, mechanisms of electronic communications, and online learning, as measures to cope with the pandemic [19].

New ways of living have had an enormous impact on people's physical, emotional, and psychological health and well-being. It is perhaps understood that there is no return to the absolute normal that existed before the pandemic [20]; there is more preparedness to move forward into the new reality and new world order, which is essential. Preparation for a new normal will be far from easy and will require arduous effort on everyone's part. The psychosocial damage that has been caused is in no way going to be fully undone; however, developing new social and life skills and enhancing the psychological preparedness quotient in social, physical, intellectual, cognitive, and emotional domains might be the only effective and relevant approach to rebuilding the fabric of social systems and accepting new reality as it dawns on us.

The article discusses the role of psychologists, especially from the standpoint of the enormity of psychological damage caused by the pandemic, and optimally using social and mental skills to effectively cope with the effects. Psychologists, therapists, and counselors may be better choices to help people develop and enhance coping skills; it needs to be acknowledged at the beginning that their role as facilitators and trainers has also changed, given the complexity of the disaster. The article indicates the role of the counseling psychologist in a post-pandemic reconstruction program and how their role and responsibilities have changed. We also discuss how counselors could adopt and incorporate standard operating procedures from military training and organizations to develop more robust coping programs. These coping programs are more likely to be most useful to special groups or high-risk groups like medical and healthcare workers, EFRs, soldiers, and adolescents. Lastly, the article will give recommendations for frontline COVID-19 warriors and EFRs on how to be better prepared to deal with unwanted stress due to the pandemic, be it dealing with sleep disorders, fear of infection, frustration, boredom and loneliness, anxiety, etc.

Reassessing the role of psychologist in crisis management

January 2022 marked two years of the world being under siege by the SARS-CoV-2. These two years have been stressful, and people have lived in fear of infection and anxiety. Some segments of the population were more vulnerable to the risk of depression and post-traumatic symptoms because of various reasons, such as proximity to infected individuals, lack of support, economic conditions, and loss of employment opportunities, to name a few. These vulnerabilities have resulted in a rise in suicide and suicide ideations [9], increased feelings of anger [10], and an increase in cases of anxiety problems [14]. These conditions and symptoms could point to serious psychological issues if not managed in time [21]. The role of a psychologist, counseling therapist, and mental health professionals in the current situation needs to be reassessed. While trained to deal with mental health issues and issues of adjustment, even for mental health professionals, the magnitude of the pandemic can be daunting. From facilitating intervention programs and providing counseling to a few individuals, the role may now have to shift to the gross or macro level, where psychological issues that a person or group of people bring into counseling or therapy sessions are likely to have a big element of uncertainty attached to it. Another big change that characterizes counseling or therapy would be how any intervention plan needs to consider that execution of the same can be a mammoth task, given the dynamics of the uncertain, ambiguous context it needs to operate. The role of a psychologist and other mental health professionals thus needs a re-assessment.

Pešić and Miljković [22] state that psychological knowledge is important in order to educate the population to

prepare for crises, i.e., the knowledge is useful in the time before the crisis – pandemic, in overcoming the crisis during the pandemic as prevention and reduction of activities during the society rehabilitation process after the pandemic.

Given the changes in the counseling or therapy landscape in the last two years, the following points need to be considered for psychologists and mental health practitioners: 1) In the absence of a similar situation, psychologists are likely to refer to similar (only moderately similar) situations like other diseases and epidemics and global events that perhaps had a similar-scale impact. Considering the high risks of contraction and contagion, psychologists choose to adopt new-age ways of working, i.e., remote counseling and therapy to minimize physical contact with clients or using telecommunication as a means to continue facilitating support and services to mental health [23]. Psychologists need to show flexibility to adopt and adapt to new ways of working. 2) In order to facilitate improvement in mental health quotient and public mental health, psychologists and counselors need to relook at the pandemic from a positive psychological standpoint. Positive psychology perhaps looks at suffering as a common aspect of human life and indicates that positive processes or traits like resilience, mental strength, willpower, and post-traumatic growth (PTG) help individuals recover and rebuild from adversity or crisis [24, 25]. Psychologists need to identify and believe in this ability to rebuild and grow after a crisis, to help others manage and cope with the adversity. 3) The situation undoubtedly qualifies for a crisis and it is typical of post-crisis problems, such as major depressive disorders, and post-traumatic disorders are likely to be on the rise [26, 27]. Thus, the counseling approach may need to be redirected to support post-traumatic stress reactions, emotional distress, and other trauma-related disruptions. Therapy or counseling approach in such challenging times needs to be reassessed to address problems and contextual limitations. 4) Psychosocial group interventions, which have proven success records, may not be possible in ways done earlier, given the need to maintain social distancing [28, 29]. These are challenges for some psychologists, especially in areas where group interventions are more appropriate than individual sessions. Psychologists will now need to identify how they can replace group sessions with other second-best alternatives according to the needs of the clients and/or patients. 5) Lastly, it is critical to understand that the role of a psychologist will be amplified and identified as one first-responder role in the pandemic and post-pandemic reconstructions [30].

While governments will continue to focus their attention on rebuilding the economy and its commercial and financial systems, none of these efforts may be fructified unless there is a mentally strong and fully functioning fleet of trained human capital that is effectively bereft of mental health issues. A psychologist's role in building strong, well-adapted human capital will continue to be important in times to come. Psychologists, counselors, and mental health professionals need to acknowledge this big change and respond in the best interest of humanity [30]. It becomes imperative for health professionals to acknowledge that although the pandemic probably was a bad experience for each individual, each individual experienced it differently. Certain stressors are particular to different groups of people within communities, such as families of vulnerable individuals and frontline workers. Emergency psychological crisis interventions should be aware of the effects and consequences of the pandemic on specific communities, and generalizations in diagnostics, therapy, or counseling approaches need to be used with caution.

The role of a psychologist has never been so important, not just for an individual but for nations and the world at large. The goals of counseling and psychological interventions become important topics of discussion. Understandably, the primary goal or objective of any counseling practice is to enhance a client's ability to cope with their life situations. In the COVID-19 world, the focus of counseling and therapy may be centered on facilitating changes in decision-making and behavioral changes which may have larger implications for a nation's effort to rebuild itself in the post-pandemic times. For instance, when a counselor helps an adolescent to develop appropriate habits of social distancing and following hygiene protocol, the implications of these interventions are likely to benefit at a macro level.

Counselor's role in facilitating resilience

Resilience can act as a factor in order to nurture PTG [31]. Crisis counseling serves an important function of helping individuals deal with the crisis by helping the clients develop adaptive skills, such as adaptive decision-making and problem-solving. Crisis counseling thus aims at bringing about behavioral changes that prove beneficial, productive, and long-term, and applicable if a similar situation arises again. Counselors and psychologists who embark on the mammoth task of facilitating cognitive and behavioral changes in the wake of the pandemic may have to incorporate some general rules in their approach while continuing to customize therapy to the client's subjective needs and context. These rules may include the following: 1) Safety first rule: As a part of interactions with the clients, the counselor can check what safety measures the client is taking. Psychologists can play an active role in correcting irresponsible behaviors or clarifying information that could be detrimental to the client's well-being and others around them. Counselors and psychologists need to exhibit appropriate hygiene and safety protocols and behaviors themselves to bring about behavioral changes among the clients. 2) Help the client deal with distress: Training in counseling equips professionals to develop listening, summarizing, and rephrasing skills. These skills are likely to be most useful when clients disclose their distress and emotional experiences resulting from changes in life situations due to the pandemic. 3) Facilitate healthy ways and coping skills to deal with negative emotions. Negative emotions, emotional disturbances, and fear of contracting the disease that surfaced during the pandemic are likely indicators of serious psychiatric diseases in the future if not

treated in the present [32, 33]. Counselors should focus on facilitating rephrasing or restructuring of emotional experiences such that these do not become great problems in the future. 4) Support enhanced communication among close relations and social interactions: Help clients identify the importance of open and supportive communication methods in the family, all within the limits of safety protocols. There is research evidence suggesting a positive relationship between social support, or social coping, and psychological well-being, especially in dealing with post-traumatic stress [34–36]. 5) Help clients cope and deal with life situations and suggest some appropriate ways to enhance coping.

The SAP-DISC model

Building on general guidelines for counseling and psychologists to deal with the effects of the pandemic [37], as well as the authors' previous works [38–42], we propose this model, which includes some key techniques and competencies specific to challenges posed by the pandemic. Some of these techniques are the result of first-hand experiences of working in high-stress situations like military operations and personal experiences. The model includes the concepts of self-efficacy [43], adaptive resilience [44], PTG [45], decompression [46], impulse control [47], social consciousness [48], and cognitive flexibility [49]. The model can be remembered using the acronym SAP-DISC.

Self-efficacy

One way in which the pandemic has eroded through the pool of human strengths is the area of self-efficacy, that is, the belief in oneself to be able to carry on.

Bandura [43, 50] postulated that self-efficacy emerges from four major sources, namely enactive mastery experiences, vicarious experiences, verbal persuasion, and physiological reactions. Bandura [50] has defined self-efficacy as "...[the] beliefs in one's capabilities to organize and execute the courses of action required to produce given attainments" (p. 3). That is about what one considers attainable with the skills one possesses and not about the abilities and skills one actually possesses [43]. Boe and Bergstøl [51] reported that when military officers were forced to choose which factor in total had the largest impact on self-efficacy, a clear majority of respondents indicated the factor of enactive mastery experiences.

High levels of anxiety arising from the uncertain nature of the pandemic may result in lowered self-efficacy [52, 53]. During the early parts of lockdown restrictions, people who believed they could follow the guidelines laid down by governments and global healthcare organizations were able to absorb the shock of significant changes to their lives. People who believe they are able to swim through the rough tides are likely to have contingency plans and very well-defined goals that guide their actions. Individuals high on self-efficacy beliefs are capable of evaluating whether their actions are in line with the beliefs and goals they have set for themselves. Research studies support the relationship between positivity, emotional self-efficacy, and ability to manage adversities [54]. Beliefs of self-efficacy help in reducing anxiety and depressive symptoms and serve as psychological assets for optimal functioning [55–57]. Counselors can help their clients rebuild self-efficacy beliefs. Considering that the load on resources has been increasing, along with limitations such as decreased social support and social distancing, may emphasize the need for individuals to help themselves.

Adaptive resilience

Adaptive mindset and adaptive thinking are likely to be the key to the successful management of a high-risk situation with high stakes like the pandemic. Resilience building must emphasize the importance of recovery and adaptation in the aftermath of disruption [44]. Resilience building should be looked at as a long-sustaining endeavor, meaning clients should develop habits and skills that help them face not just the pandemic but pandemic-like events with resilience and grit in the future. Counselors should help their clients transition from resilience to adaptive resilience. Adaptive resilience, in a nutshell, refers to an ability to recover from adversity and adapt but also thrive [38]. Adaptive resilience is thus the capacity to remain productive, behave and emote in line with set goals and objectives whilst absorbing disturbances and doing so with integrity. An adaptive resilience mindset would likely result in definite sustainable behavioral changes. Adaptive resilience has proven to be an effective competency, especially in the case of coping with complex crises. For instance, Walker and Nilakant [58] found that during natural calamities like the Christchurch earthquake in New Zealand in 2011, an adaptive mindset with an emphasis on sustaining and thriving and finding new opportunities helped people come out of the situation stronger.

Post-traumatic growth attitude

PTG attitude is defined as an emphasis on positive psychological changes that occur following highly stressful and demanding life situations [59]. Positive transformation is known to include three domains: personal strengths, relating to others, and appreciation of life – openness to new possibilities and spiritual changes [45]. Perceptions or thoughts about PTG resulting from experiences of a stressful event like the pandemic are likely to result in better-adjusted individuals [60, 61]. Counselors can help their clients' PTG attitude by improving relationships with significant others, appreciating their lives more, and perhaps developing a spiritual perspective if it should be beneficial [30]. Growth arises from the way the event is processed, not from the event itself, and leads individuals to recognize their vulnerability and what they can and cannot control and to reassess their personal priorities [31]. Thus counselors and psychologists should help clients focus on what positive can come out of adversity beyond obvious negative effects of the pandemic. Research evidence suggests that people across all age groups who have experienced traumatic events are able to identify positive ways in which they can re-evaluate stressful situations, and this ability to re-evaluate situations positively leads to improved mental

health and well-being [62, 63]. Given that the pandemic has been a negative, dampening event, exploration of possible PTG perspectives may redirect their energies to rebuilding their life situations [64].

Decompression

A conceptual understanding of the concept of psychological decompression is mostly mentioned in military literature.

According to Piotrowski et al. [46], decompression aims at providing us opportunities for the 9 R's, namely, rest, relaxation, recognizing own and others' achievements, reducing the stigma of post-pandemic adaptation problems and challenges, reflecting, realizing the need for self-regulation as well as self-reliance at the national level, reintegrate, reboot and reconstruct a new world order in the post-pandemic era, and resume a new way of a balanced life.

In the military context, decompression refers to a period for the soldiers between military operations and homecoming [65]. Decompression is viewed as a period of transition between a high-stress operational environment with heightened emotional states, guilt of survival, high tension, extreme alertness, and a home environment perceived to be relatively less stressful [66]. Acknowledging negative emotions becomes imperative and strategies to manage these negative emotions must be such that they do not interfere with well-being. A period of psychological decompression with a focus on rest and cognitive and emotional reappraisal can prepare soldiers to adapt to the environment at home. Crisis counselors can help clients use techniques such as sufficient rest and relaxation, developing self-regulated behaviors, developing psychological equilibrium, etc., as part of a decompression intervention. Counselors should encourage clients to balance their personal and professional lives and take some time off to deal with grief or trauma that they may be experiencing.

Taking a closer look at the caregivers themselves during the COVID-19 pandemic, Luo et al. [67] reported that mindfulness decompression therapy had a positive effect on mental health and reduced job burnout among nurses working in the pandemic frontline. Lefèvre et al. [68] investigated healthcare staff and found that a program and a space for relaxation and support for hospital caregivers by hospital caregivers offered help in both decompression and relaxation. Acknowledging and managing residual negative emotions and moods could prepare both clients and EFRs to rebound and adapt to the new reality in post-pandemic times.

Impulse control

Impulse control is defined as the ability to modify or override one's inner responses as well as interrupt undesired behaviors [47, 69]. During the pandemic, many instances have prompted impulsive actions and emotions, especially because of the ambiguous nature of the pandemic. For instance, the impulse to break protocols of lockdown, social distancing, or hygiene behaviors may have passed through the minds of many. The temptation to fall prey to the impulse may cost an individual dearly and place them further into a zone of vulnerability. On the other hand, Yule et al. [70] reported an increase in impulse control behaviors during the COVID-19 lock-down in the UK. They studied people with Parkinson's disease to see whether this group might handle impulse control in a different way as compared to other groups in the society [70]. In general, there is a need to understand and learn about self-control or impulse control during taxing situations for most people.

Self-control or impulse control may serve to regulate undesirable mental health consequences [71, 72]. With fewer resources being available for consumption in times of a pandemic, self-control may be regarded as a psychological resource to manage the effects of the pandemic. Self-control, or impulse control, is known to be associated with lower degrees of depression and anxiety [73]. Closely connected to the concept of self-control is the concept of self-regulation. Self-regulation is defined as the process of purposefully directing one's actions, thoughts, and feelings toward a goal; self-regulation skills include goal-directed behavior and allow us to delay gratification in the short term to achieve desired outcomes in the future [74, 75].

Janiri et al. [76] showed that having had COVID-19 may be related to a high likelihood of psychological distress in advanced-age people, and this may, in turn, be associated with impaired emotional regulation and higher scores on depressive and cyclothymic temperaments.

Learning how self-regulation behaviors are influenced by emotion regulation is likely to generate important new insights into both processes, i.e., goal-directed behavior and desired outcomes. That may lead to a better understanding of how people can optimally function in challenging environments by controlling their emotions and, consequently, an insight into their behavior to conform to norms and protocols even in emotion-arousing situations [77–79].

Depressive conditions and anxiety disorders are linked to other psychological disorders. The relationship between impulse control and depression should be the focus of counseling and therapy, especially for clients who could benefit from developing good habits or who show vulnerability to depression and anxiety problems [76].

Socially congruent behaviors

Behavioral modifications and cognitive adaptability have been part of counseling objectives since time immemorial. However, there is a renewed need for behavioral modifications and cognitive reappraisals in the pandemic. The responsibilities of counselors and psychologists now may need to include an emphasis on socially appropriate or responsible behaviors. Behavioral modification programs need to help clients develop behavior and cognitive patterns that benefit not only themselves but also the society at large or reduce the risks of infection and contagion for others. Additionally, it may be required to focus on lifestyle changes. Stress, depression, and reduced social interactions may lead to overeating, alcohol abuse, and engaging in maladaptive behaviors [80-82]. Frustration arising from home isolation or quarantine

may enrage people and mislead them to break protocol or engage in inappropriate behaviors. The consequence of such behaviors can be detrimental to others.

While focusing on treating stress, frustration, and sorrow at an individual level, a counselor needs to ensure that any intervention plan considers the implications of plans on a larger populace and does not allow for maladaptive behaviors in order to help the individual.

Cognitive flexibility

Cognitive flexibility refers to a person's ability or readiness to selectively switch between mental processes to generate appropriate behavioral responses and enhance an individual's resilience in the face of a stressful situation [83, 84]. Home isolation and compromised social interactions are likely to affect a person's ability to think clearly or differently. Counselors may be required to help clients identify these gaps in cognitive processing and help them re-evaluate the situation. Cognitive flexibility will enable clients to reframe the situation as not being fully in one's control and reconsider their behaviors. Cognitive flexibility has been reported to be a potential protective factor during the COVID-19 pandemic [85].

While the situation may continue to be challenging, dynamic, and shrouded in ambiguity, cognitive rephrasing and reappraisal of thinking or cognitions are likely to result in adaptive behaviors.

Demmin et al. [86] reported that both mental and physical training with meditation and aerobic exercise improved mental health and well-being in teachers during the COVID-19 pandemic. Participants also reported increased subjective ratings of executive functioning, working memory, cognitive flexibility, and fewer sleep disturbances.

Cognitive flexibility could include the ability to think creatively and flexibly through situations, thus developing more adaptive problem-solving patterns. Additionally, counselors need to focus on positive thinking. There is evidence suggesting that developing positive emotions and thinking fundamentally changes how the human brain works to broaden cognitive awareness. That enables greater information intake that, in turn, helps people enact and build resilience and social connectedness that can help individuals cope with adversity [87].

SAP-DISC is a model developed with the intention of helping counselors and psychologists help themselves and their clients better deal with the pandemic. The model focuses on some of the important adaptation techniques to buffer, reinforce, or transform the effects of the pandemic. Buffering effects of the pandemic imply using positive emotions or strengths to diminish the negative psychological effects of the pandemic [88, 89]. Techniques that focus on reinforcement use positive emotions and relationships (positive refocusing, positive reappraisal), and these techniques can be directed to maintain psychological well-being despite the crisis [90].

Luo et al. [91] found that specific resilience behaviors, including establishing a supportive social network, relying on a moral compass, and using cognitive flexibility, were techniques that medical students commonly used to cope with traumatic experiences during the COVID-19 pandemic. However, as shown by Piotrowski and Wirkus [92], people may experience burnout in a similar way but at the same time differ in the preferred style of coping with stressful situations.

Taking all this into account, some of the techniques in the SAP-DISC model can be used to transform the current situation into growth opportunities, but the techniques may also have to be tailored to each individual or group.

Helping specific groups

Some effects of the pandemic, such as separation from loved ones, loss of freedom, vulnerability to infection, disadvantaged groups, and feelings of helplessness, affect certain groups more than others [8]. That is not to say that the general population groups are not affected by risks, but it simply means that some groups are more vulnerable and need special attention to preserve these forces.

Emergency first responders

EFRs are a high-risk population group consisting of subgroups like doctors, nurses, medical professionals, the police, and firefighters, who are at the highest risk of contagion due to the nature of their jobs as well as occupational stress associated with jobs [12, 93]. The pandemic situation has interfered with and added layers of challenges to their professional and personal lives, with some apparent outcomes seen in an increase in cases of anxiety, depression, and PTSD. Psychologists and mental health professionals have a key role to play by actively participating in the overall intervention process for psychological distress, such that interventions may be mobilized in a timely fashion [94]. Psychological crisis intervention can, in general, focus on two aspects. One is the intervention for fear of contagion among special groups that have closest proximity to the SARS-CoV-2 and patients who have contracted the virus, and the second is intervention for difficulty in adapting to different environments at work and home. Tapping on the emotional stress and discomfort of highly vulnerable individuals should become an important basis for psychological intervention [95]. Two additional considerations for these groups are important: psychological interventions for their families and caregivers as well as reducing the ostracism and neglect that these groups have had to suffer during the early phases of the pandemic.

Geriatric population

The geriatric population and underlying diseases are real challenges in controlling the spread of the infection [96]. This population faces special risks of the pandemic owing to conditions like compromised physical health, co-morbidity conditions, immune system malfunctions, fears, and anxiety inherent in old age [97, 98]. These populations are vulnerable and need special attention in the form of social support interventions and mitigating the lack of preparedness of these groups. For these groups, social interactions and social sup-

port are important, adopted within the limits of government-imposed safety protocols. Counselors and psychologists need to show appropriate sensitivity to the needs of this population. Counselors may need to help older clients build a sense of respect and dignity to maintain a healthy mental status among the elderly [99]. Loneliness is a common problem among the older populace, especially in those who live alone or who are grieving the loss of loved ones. The pandemic has especially been a challenging period for such people with basic and social resources becoming difficult to access. Counselors and mental health professionals must estimate resource availability and consider how the absence of resources may be mitigated for the geriatric population. In order to help build resilience among older people, develop coping skills, and reduce loneliness, a counselor could promote cognitive behavioral therapy, physical activity, greater social and community connectedness, compassion training, and evaluate spiritual approaches [100, 101]. EFRs and their families need to be provided with psychological support and assistance whenever needed. Debriefing sessions, which help in releasing mental tension and pressure arising out of long work hours, uncertain work environments, loss of loved ones, fear of infections, and social disconnectedness experienced by EFRs, should be made available [46]. Psychological stress and trauma cases are known to be prevalent after epidemics and pandemic events, especially stress arising from stigmatizing attitudes, in particular toward health professionals who are in daily contact with the risk of infection [15]. Psychologists working with EFRs need to be sensitive to the special needs of these groups and design interventions which contribute to emotional and psychological well-being.

Soldiers and military personnel

A pandemic situation like COVID-19 takes a toll on emotional health and tests the psychological resilience of most of the populace. Some population groups may experience symptoms and effects just like other groups, but the magnitude of the impact of the pandemic on these populations affects a nation at large. Military organizations provide soldiers with psychological support and resilience training, which focus on managing stressful events on the battlefields, during peace-keeping operations, and life in deployment areas, mostly away from families [39, 40].

Military training emphasizes the role and value of group cohesion, team camaraderie, and interpersonal and team communication to develop coping mechanisms [102–104]. However, interpersonal interactions have been significantly affected due to the pandemic restrictions. Counseling facilities that were earlier available at military medical facilities may be out of access due to restrictions imposed on movement. Military families and extended communities provide a great sense of support to military personnel. Now, with military forces being isolated into gated cantonments (with the intent of force preservation), social interactions and social support are compromised [105]. Many soldiers have spent a long time isolated at home during the pandemic and are directly expected to return to their units/sub-units. This abrupt transition may be stressful, considering the level of alertness and preparedness required in the operational areas. Soldiers may experience fear and anxiety when in quarantine, which harms their mental health in the absence of immediate intervention. Thus, counselors working alongside soldiers need to be aware of the special needs of this group and develop intervention plans that allocate attention and resources for individual-oriented resilience training. These interventions are likely to focus on evaluating the psychological states of these special population groups, providing online or telephone counseling services, helping them monitor their well-being regularly, and helping them access care and encouragement in the military community in line with safety protocols that may be imposed. These support plans are likely to mitigate, at least partially, the absence of normal medical and psychological care options and help soldiers and military personnel be mentally prepared for the call of duty whenever they are likely to be deployed back into operational areas [106].

Children and adolescents

The COVID-19 threat has posed short-term as well as long-term psycho-social and mental health implications for children and adolescents. The unprecedented effects of the pandemic have resulted mainly from measures employed to curb the spread of infection, such as social isolation, maintaining distance, restricted movement, and loss of physical interaction [107, 108]. Some of the research suggests that the pandemic could prove more detrimental to the well-being of children and adolescents compared to adults [87]. Some symptoms, like increased irritability, inattention, and clinging behavior, have become apparent among children [109]. These symptoms could be indicative of serious mental disorders like depressive disorders, anxiety disorders, and others if not treated immediately. When schools and colleges shut down suddenly, around March 2020, adolescents suddenly experienced a huge change in their life situation, which they were not prepared for. That has negatively impacted a significant section of the adolescent population around the world [110]. Being confined to homes, children, and adolescents have experienced anxiety associated with uncertainty and disruption in their education, physical activities, and opportunities for socialization [111]. Some research studies have reported that children have expressed lower levels of affect for not being able to play outdoors, not being able to meet friends, or concerns about not being allowed to engage in in-person school activities [110, 112]. As a therapeutic intervention, psychologists could be required to particularly pay attention to improving sleep [113], eating habits, improving physical activities, and regulating the use of social media and the internet while working with children and adolescents [114, 115].

Some recommendations for prevention work (especially with adolescents) are training in assertive communication, strengthening self-confidence and self-esteem, training in emotional intelligence, adoption of anger management techniques, adequate ways of dealing with problems, constructive conflict resolution, and the like [116].

While working with these special groups, counselors can identify if children and adolescents have the correct information regarding safety protocols to be followed and compliance with measures undertaken to curb the spread of infection [117, 118]. In the case of children and adolescents, counselors and mental health professionals also need to seek information and observations about their mental health from teachers and parents. Psychosocial intervention programs for children and adolescents need to focus on building resilience through the expression and regulation of emotions, encouraging children to engage in meaningful and relaxing activities, and thus develop alternatives to risky behaviors like overindulgence in using the internet, overeating, sleep imbalances, etc. [119]. Counselors should additionally encourage parents of children and adolescents to continue monitoring the overall health signs and report if they observe their children's behaviors or emotions being unusual or deviant.

Impact on psychologists

Psychologists emerged as important health professionals out there to save the world from the clutches of the vicious pandemic. However, they are as vulnerable, if not more, than any individual to be affected by the suddenness, uncertainty, and ambiguity of the pandemic effects and consequences of being thrown into a storm of emotional disturbances and psychological problems. As such, there is a need to give them different techniques to cope with these possible negative effects [46, 67, 68]. The American Counselling Association has proposed that counselors and mental health professionals follow guidelines for self-care [120]. These guidelines include practical and necessary recommendations like maintaining contact with social and professional groups that can facilitate a soundboard to help psychologists cope with their problems and engage in peer discussions to understand varied approaches to deal with the vices of the pandemic. Professionals who provide in-person counseling are advised to adhere to safety protocols, maintain social distancing, and enforce hygiene and safety standards for their own and client safety.

Psychologists and counselors are essential forces and assets, and special attention to safeguard this group of professionals needs to be put in place. Psychologists, just as medical professionals, EFRs, and the police, belong to the groups that need to be protected. Psychologists need to play a crucial role for nations to rebuild their strengths after the COVID-19 pandemic.

Conclusion

Counseling is a safe space where a counselor listens and understands a client's story from their perspective and makes an effort to facilitate the transition in the direction that the client desires. Counseling people through present-day crises like the COVID-19 pandemic is no easy job; however, the role and importance of psychologists and counselors are now seen as crucial to helping communities and Nations reinstate the social fabric that was challenged. Clients come to the counseling service with high distress and concerns about uncertainties in their life situations that the stressful situation has resulted in. Providing them with emotional support helps clients feel less distressed and begin focusing on important aspects of their lives. Though counselors and mental health professionals are not expected to have all the answers in an ever-complex situation, counselors are the key sources of information on how to use adaptive approaches to deal with the ordeal at hand. As we are slowly retrieving ourselves from the COVID-19 pandemic, it is fair to say that the role of a psychologist and mental health professionals has been both challenged and valued at the same time. With great respect for the craft, greater awareness of the responsibility that lies with psychologists and their actions should also arise.

The quintessential qualities of counselors and psychologists, such as empathy, flexibility, and contextual understanding, are likely to be put to the test, given the complex nature of the pandemic. Additionally, a counselor's ability to preempt signs of emerging disorders, ability to understand the contextual needs and challenges of the clients, counselor's own sense of resilience, and personal situations are likely to decide the course and outcome of psychological interventions and recovery process.

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Conflict of interest

The authors declare no conflict of interest.

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Possibilities of stroke care improvement in Serbia: consensus document for the prevention, treatment, and rehabilitation of stroke in Serbia

Mogućnosti za unapređenje zbrinjavanja bolesnika sa moždanim udarom u Srbiji: konsenzus dokument za prevenciju, lečenje i rehabilitaciju bolesnika sa moždanim udarom u Srbiji

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Introduction

A stroke represents an urgent, life-threatening, neurological medical condition that requires immediate attention. Over the past few decades in the field of neurology, especially stroke, we have witnessed the complete recovery of the nervous system after severe clinical manifestations, mainly ischemic but also haemorrhagic strokes. However, stroke is the second cause of death in the Republic of Serbia (RS) and the main cause of disability in adults [1–4].

Globally, over the past three decades, the total number of years lived with disability (Disability-adjusted Life Years, DALYs) due to stroke has significantly increased (from 91.5 million in 1990 to 125 million in 2019, an increase of 33.5 million) [5–7]. There are different trends in countries with high income and those with low to middle income, to which RS belongs. The large increase in the global burden of stroke is not only due to population growth and aging but also due to a significant increase in exposure to important risk factors, such as high body mass index, environmental pollution, high blood glucose levels, high blood pressure, alcohol consumption, physical inactivity, kidney dysfunction, and high temperatures [5–7].

Although there has been a general reduction in standardized incidence, prevalence, mortality, and DALY rates, low to middle-income countries carry the highest percentage of the stroke burden [5, 7]. A large number of strokes could be successfully prevented, representing a significant potential for reducing the burden of stroke, including a significant reduction in long-term consequences. Given the high morbidity and mortality rates, stroke represents a significant medical and socioeconomic problem. The aim of this paper was to define the healthcare system's actions for achieving the best treatment outcomes for stroke patients in RS.

To achieve the best possible health outcomes, it is important to establish priorities and principles for stroke care, including diagnostic and therapeutic protocols spanning from primary prevention to long-term rehabilitation, with a focus on the clinical path as a fundamental component of a doctor's approach. As a result, the Association of Neurologists of Serbia (ANS) approved the development of the "consensus document for the prevention, treatment, and rehabilitation of stroke in RS" [8]. This document offers a comprehensive recommendation for stroke care in RS and serves as a practical guide for clinicians and healthcare professionals, taking into account patients' risks, complications, and evidence-based (EB) approaches to diagnosis and treatment [8]. In 2021, a study was initiated by the ANS and the regional, international consulting agency for health policy and health economics projects, "ZEM Solutions", with the aim of providing a detailed understanding of the current clinical practice for treating stroke patients in RS. To develop the

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Consensus Document (CD) [8], a working group was appointed by the ANS presidency. The working group included major healthcare institutions for stroke care in RS, such as the University Clinical Center of Serbia (UCCS), the University Clinical Center of Vojvodina (UCCV), the University Clinical Center Niš (UCCN), the University Clinical Center Kragujevac (UCCKg), the Military Medical Academy (MMA), the Special Hospital for Treatment of Cerebrovascular Diseases "Sveti Sava", reference Special Rehabilitation Hospitals, and consulting agency "ZEM Solutions".

The CD [8] is based on principles outlined in the National Stroke Guidelines of RS [9], incorporating the best practices observed in the local clinics that adhere to the standards of Good Clinical Practice (GCP). During the development of this document, the latest European and American guidelines for managing stroke patients and stroke prevention were consulted [10–16]. The CD was developed in accordance with the goals of the European Action Plan for Stroke 2018–2030 [17]. Particular emphasis was given to reviewing previous knowledge and clinical experience to ensure the document is tailored to the current state of the healthcare system in RS and enables optimal care for stroke patients.

The working group presented a preliminary draft of the consensus paper to the board of directors of the National Stroke Association of Serbia (NSAS) and the Ministry of Health working group responsible for formulating a national stroke strategy in November 2021. After receiving feedback, the working group addressed the comments and made necessary revisions. The final version was approved in May 2022 during the NSAS Board of Directors meeting, and the complete document has been published and is now available on the ANS website. The recommendations provided, along with the corresponding levels of evidence from the American Stroke Association [15], were used throughout the development of the document.

Recommendations for stroke care improvement in Serbia

The following is included in the CD [8]: prehospital care, emergency assessment and treatment with intravenous and endovascular (EV) therapy, secondary prevention measures starting from initial hospitalization, and primary prevention and rehabilitation measures.

For each of the aspects of stroke care, the recommendations listed in the CD [8] are presented in the following text.

Primary prevention (chapters 3 and 11)

There is significant potential for reducing the burden of strokes in RS by preventing and effectively treating a large number of cases. Roughly 80% of strokes may be averted through the screening and controlling of known risk factors, including actions such as: enhancing hypertension regulation, smoking cessation, preventing diabetes, controlling cholesterol levels, increasing the use of anticoagulants for atrial fibrillation (AF), and eradicating excessive alcohol consumption [18, 19]. As part of primary prevention, various screening models are organized to proactively identify individuals with an increased risk of stroke. To prevent stroke in eligible patients with AF requiring oral anticoagulants (OACs), the European Society of Cardiology (ESC) recommends non-vitamin K antagonist OACs (NOACs) over vitamin K antagonists (VKAs), except in cases involving mechanical heart valves or moderate to severe mitral stenosis [12]. Primary and secondary stroke prevention are addressed by general practitioners, internists, and physicians from various specialties.

Stroke care organization (chapters 10 and 11)

Whether ischemic or hemorrhagic, stroke is a medical emergency that requires urgent attention and offers a high probability of functional recovery. To ensure the timely administration of stroke therapy, it is essential to promptly recognize symptoms, obtain immediate assistance from emergency medical services, and provide effective treatment upon admission to the hospital [20]. The location and availability of emergency medical services are of utmost importance as they play a critical role in ensuring prompt, efficient, and expert triage. That has a significant impact on achieving the established standards for stroke treatment. Air ambulance services greatly reduce the disparities created by the distance of services and transfer between them [21]. A crucial recommendation for enhancing stroke treatment is the organization of hospital services to offer care in specialized stroke units. This type of care is shown to substantially lower mortality rates and reduce the disability burden in stroke patients compared to conventional care in general hospital wards [22]. Improving technical and transportation capabilities can lead to a further reduction in the time it takes to travel from the onset of clinical symptoms to arrival at hospital emergency departments and stroke centers. The development of telemedicine can provide an opportunity for primary healthcare physicians without access to stroke units to seek advice from neurologists and neuroradiologists in acute stroke care and rehabilitation [23]. To ensure that all patients have equitable access, it is essential to increase the number and capacity of comprehensive stroke units (also known as stroke centers or advanced stroke units). In addition, it is necessary to establish a fully operational network of institutions dedicated to stroke care throughout RS and extend the advanced stroke unit network to include major regional general hospitals that possess the necessary resources, such as neurosurgeons, vascular surgeons, 24-hour radiological diagnostics, and intervention specialists.

Hospital treatment of acute stroke (chapters 6 and 11)

Early stroke treatment is essential for reducing the likelihood of complications during the early stages of stroke. Treatment protocols should be based on EB best practices. The administration of *iv* thrombolytic therapy (IVT) using alteplase has been shown to be effective and safe for ischemic stroke (IS) when given within 4.5 hrs of symptom onset and is currently the standard of care. For certain patients who are

eligible for mechanical thrombectomy, tenecteplase may be used instead of alteplase within this therapeutic window. For patients experiencing acute IS and significant intracranial vessel blockage, mechanical thrombectomy (MT) is a recommended treatment option within 6 hrs of symptom onset. In selected cases where multimodal imaging techniques are used, MT may be considered within 16–24 hrs after the onset of symptoms. A multidisciplinary team in stroke units is recommended for the treatment of stroke patients, as it has been demonstrated to improve outcomes and reduce mortality in individuals with acute IS. The hospital system needs to be organized in a manner that brain imaging diagnostic methods are performed within 20 min of arrival at the emergency department for a minimum of 50% of patients who may be eligible for IVT and/or MT.

Secondary prevention (chapters 8 and 11)

The neurology department has a key role in implementing EB medicine, GCP, and national guidelines for secondary stroke prevention. Neurologists should play an active role in secondary stroke prevention alongside general practitioners, internists, and other specialists. Patients with non-cardioembolic IS or transient ischemic attack (TIA) are recommended to use oral antiplatelet drugs to reduce the risk of recurrent stroke and other cardiovascular events, while oral anticoagulant therapy is not advised. An integral component of secondary prevention of IS is the management of hypertension, which involves frequent monitoring of blood pressure and achieving recommended target values of < 130/80 mmHg. As part of secondary stroke prevention, it is recommended to regulate blood glucose levels through lifestyle changes and individual pharmacological therapy. Administering statins is recommended for managing patients with acute IS or TIA who have high cholesterol levels or coronary artery disease. Patients who smoke and have recently experienced TIA or stroke are advised to quit smoking. Patients with recent TIA or non-disabling IS and ipsilateral severe (70–99%) carotid stenosis are advised to undergo carotid endarterectomy (CEA) within two weeks if the perioperative risk of morbidity and mortality is estimated to be less than 6%. Carotid angioplasty and stenting is indicated as an alternative to CEA for symptomatic patients with average or low risk of complications associated with EV intervention when the luminal diameter of the internal carotid artery is reduced > 70% according to noninvasive imaging (ultrasound) or > 50% according to angiography by the North American Symptomatic Carotid Endarterectomy Trial criteria [24]. In secondary prevention of IS, OACs are recommended for all patients with AF. For patients who meet the criteria for NOAC therapy and have no strict contraindications for OAC use, NOACs are preferred over VKAs [12, 16].

Rehabilitation (chapters 7 and 11)

Rehabilitation should be initiated as soon as possible as the main contribution to a positive outcome for stroke survivors and their families. An interdisciplinary approach is considered the best approach for stroke patient rehabilitation.

Long-term care – Life after stroke (chapters 9 and 11)

This is one of the most important aspects of support for stroke survivors and their families.

Our study provides a summarized overview of recommendations and findings for the prevention, treatment, and rehabilitation of stroke in RS with the aim of providing the best possible treatment outcomes for patients in RS. Stroke is a major national health priority, and the importance of our previous publication [8] is significant given the discouraging epidemiological data on stroke in RS [2–4]. It remains the primary cause of death in hospital settings and the second leading cause of overall mortality in the population [2–4]. Improving stroke prevention programs would undoubtedly lead to a reduction in the incidence and morbidity of stroke from the first occurrence. However, two significant barriers to implementing thrombolysis/thrombectomy protocols are high initial costs and a lack of appropriate infrastructure and healthcare resources, resulting in lagging in the goals set by the European Stroke Organization [16]. Addressing these issues will require improvements in the organizational aspects of stroke care, including ensuring equal access to stroke units by increasing their number and capacity and establishing a functional network of stroke care facilities across RS. Based on the data currently available, individuals residing in rural regions of some countries have a lower probability of a favorable stroke outcome compared to those living in urban counterparts [21]. This problem can be solved by improving technical and transport capabilities, introducing Helicopter Emergency Medical Service, and developing telemedicine. The introduction of NOACs to the reimbursement list covered by mandatory health insurance would also contribute to improving stroke prevention in RS [25], as is the case in other reference and neighboring countries such as Croatia [26] and Slovenia [27].

Life after stroke historically has been considered an aspect of rehabilitation. Today, it is acknowledged that immediate attention is necessary and that life after stroke merits recognition in its own right. Due to life after stroke only recently being considered a separate entity, relatively few research studies cover the entire lifespan, those that do focus on reporting outcomes from rehabilitation, and there is little reference to life after stroke in national guidelines [17]. As such, research and recommendations covering life after stroke represent a significant opportunity for stroke care improvement, not just in RS but internationally.

Central and Eastern Europe have led the world in developing a framework for audit and feedback mechanisms, which have proved effective in improving the uptake of EB stroke treatments [28]. Quality monitoring of stroke care using such mechanisms plays an essential role in the timely identification and quantifications of stroke care provision [29] and the communication of evolving issues to those monitoring the registry.

RS currently has four hospitals and nearly 9,000 patients enrolled in the Registry of Stroke Care Quality [30]. In addition

to providing vital information on stroke quality, such registries also form a key evidence source for local cost and burden of stroke studies [31]; it can, therefore, be recommended that this registry be expanded to cover all stroke patients in RS.

Our study was designed to demonstrate the importance of adhering to protocols such as the CD [8] by monitoring specific parameters. That has been convincingly demonstrated through the analysis of the use of novel anticoagulant drugs and the results of mechanical thrombectomy concerning stroke recurrence, outcomes, and treatment costs. In all of these aspects, there was a highly statistically significant association between anticoagulant therapy use in stroke prevention (especially in AF) and the importance of stroke regarding outcomes and direct and indirect treatment costs [32].

In our opinion, this study contributes to understanding current stroke treatment, prevention, and rehabilitation guidelines and serves as a document for primary healthcare and advanced stroke treatment units to take action. It also highlights the importance of EB medicine to healthcare policymakers and regulators.

Conclusion

Although there have been improvements in therapy and prevention, particularly for IS and hemorrhagic stroke, stroke continues to be one of the primary causes of mortality and the primary cause of disability among adults in RS. The clear and comprehensive medical scientific evidence for stroke care shows a great need for substantial investment in stroke care in RS to alleviate the burden of stroke in the country.

Collaborative efforts from various entities, including the Ministry of Health and Social Welfare, other governmental organizations, professional associations advocating for stroke prevention and treatment, such as ANS and NSAS, scientific communities, clinical and preclinical researchers, insurance companies, and industries, are required to implement this approach effectively.

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Cytokine profile in critically ill patients and/or injured persons with secondary sepsis – influence of different pathogens

Profil citokina kod kritično obolelih bolesnika i/ili povređenih osoba sa sekundarnom sepsom – uticaj različitih patogena

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Abstract

Background/Aim. The role of the complex sepsis-related immune response has not been fully clarified and remains a subject matter of investigation. Nowadays, sepsis is considered a dynamic syndrome characterized by many, often antagonistic phenomena, ranging from hyperinflammation to anergy and immunoparalysis. The aim of the study was to determine, based on the level of pro- and anti-inflammatory mediators in critically ill patients with secondary sepsis, whether the cytokine profile differs according to the type of bacterial causative agent, as well as to assess the prognostic value regarding the outcome. The outcome measure was in-hospital mortality. **Methods**. Blood serum samples were taken from 125 critically ill patients admitted to the Surgical Intensive Care Unit with severe secondary sepsis as a consequence of peritonitis, pancreatitis, or trauma. The average age of the patients was 57.7 ± 17.3 years. Of the total number of patients, 84 (67.2%) were males, and 41 (32.8%) were females. The levels of pro-inflammatory interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, IFN-γ-inducible protein-10 (IP-10), monocyte chemoattractant protein-1(MCP-1), macrophage inflammatory protein (MIP)-1α and MIP-1β, as well as anti-inflammatory mediators IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33, were determined at three time intervals – on the day of admission (the first day) and then on the third and fifth day. The type of the bacterial causative agent was determined using standard microbiological analyses. **Results**. On the third day of measurement, significant differences in the cytokine levels regarding the nature of bacteremia were determined in all pro- and anti-inflammatory cytokines, except for IL-8. In general, the lowest levels were observed in patients with polymicrobial blood cultures. On the first and fifth days of measurement, no significant differences in the cytokine levels regarding the nature of bacteremia were found. The only significant predictor of the fatal outcome on the first measurement day was IL-17A, Area Under the Receiver Operating Characteristic (ROC) Curve (AUC) of 0.665 (95% confidence interval 0.519–0.791; $p = 0.034$) in the patients with secondary sepsis as a complication of peritonitis. **Conclusion**. According to the type of bacterial causative agent, the lowest levels of cytokines have been observed in patients with the polymicrobial blood culture. The low level of IL-17A on the first day of measurement is a good predictor of a fatal outcome in patients with peritonitis as an underlying condition of secondary sepsis. On the other hand, the levels of other cytokines correlated with the outcome only on the fifth day of measurement, and they were higher in survivors than in non-survivors.

Key words:
blood culture; critical illness; cytokines; prognosis; sepsis; treatment outcome.

Apstrakt

Uvod/Cilj. Uloga kompleksnog imunskog odgovora u sepsi i dalje nije do kraja razjašnjena i ostaje predmet istraživanja. Danas se smatra da je sepsa dinamički sindrom koji karakterišu mnogi, često antagonistički fenomeni, u rasponu od hiperinflamacije do anergije i imunoparalize. Cilj rada bio je da se na osnovu nivoa pro- i anti-inflamacijskih medijatora

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kod kritično obolelih osoba sa sekundarnom sepsom utvrdi da li se citokinski profil razlikuje u odnosu na vrstu bakterijskog uzročnika, kao i da se proceni prognostička vrednost ovog nalaza u odnosu na ishod. Mera ishoda bila je hospitalni mortalitet. **Metode.** Uzorci seruma periferne krvi uzeti su od 125 kritično obolelih bolesnika primljenih u hiruršku jedinicu intenzivne nege sa potvrđenom teškom sekundarnom sepsom kao komplikacijom peritonitisa, pankreatitisa ili traume. Prosečna starost bolesnika bila je 57,7 ± 17,3 godina. Od ukupnog broja obolelih, 84 (67,2%) su bili muškarci, a 41 (32,8%) žene. Određeni su nivoi pro-inflamacijskih interleukina (IL)-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, faktora nekroze tumora (TNF)-α, interferona (IFN)-γ, IFN-γ-inducibilnog proteina-10 (IP-10), monocitnog hemoatraktantnog proteina (MCP)-1, inflamacijskog proteina makrofaga (MIP)-1α i MIP-1β i anti-inflamacijskih medijatora IL-4, IL-10, IL-13, IL-27, IL-31 i IL-33 u tri vremenska intervala – na dan prijema (prvi dan) a potom trećeg i petog dana. Standardnim mikrobiološkim ispitivanjima određena je vrsta bakterijskog uzročnika. **Rezultati.** Trećeg dana merenja ustanovljene su značajne razlike u nivoima citokina u odnosu na prirodu bakterijemije kod svih pro- i anti-inflamacijskih citokina, osim kod IL-8. Generalno, najniži nivoi utvrđeni su kod bolesnika sa polimikrobnom hemokulturom. Prvog i petog dana merenja nisu nađene značajne razlike u nivoima citokina u odnosu na prirodu bakterijemije. Jedini značajan prediktor fatalnog ishoda prvog dana merenja bio je IL-17A, *Area Under the Receiver Operating Characteristic* (ROC) *Curve* (AUC) 0,665 (95% interval poverenja 0,519–0,791; $p = 0.034$) kod bolesnika sa sekundarnom sepsom kao komplikacijom peritonitisa. **Zaključak.** Prema vrsti bakterijskog prouzrokovača utvrđeno je da su najniži nivoi citokina bili kod bolesnika sa polimikrobnom hemokulturom. Niska koncentracija IL-17A prvog dana merenja je dobar prediktor smrtnog ishoda kod bolesnika sa sekundarnom sepsom koja je nastala kao komplikacija peritonitisa. Nasuprot tome, nivoi ostalih citokina korelisali su sa ishodom tek petog dana merenja i bili su viši kod preživelih, u odnosu na umrle bolesnike.

Ključne reči:
bakteriološke tehnike; kritična stanja; citokini; prognoza; sepsa; lečenje, ishod.

Introduction

There is a complex immune response characterized by a dysfunction of neutrophils and monocytes, the key cells of the innate immune response, activated in critically ill surgical patients with secondary sepsis, often occurring as a consequence of severe acute pancreatitis, peritonitis, or trauma [1]. In some patients, the anti-inflammatory response is prevalent. The particular problem with the treatment of critically ill patients with sepsis is the fact that a large number of them stay for a long time in the intensive care unit (ICU) with dysfunction of various organs – basically, their condition is chronically critical. Their clinical process is characterized by very persistent catabolism with malnutrition, poor wound healing, immunosuppression, and recurrent infections. Thus, a special entity has been proposed, namely, a new Persistent Inflammation, Immunosuppression, and Catabolism Syndrome – PICS [2, 3]. The study by Boomer et al. [4] has shown that the patients who died from sepsis had had biochemical, immunohistochemical, and phenotyping signs pointing to immunosuppression. According to their study, to determine the association of sepsis with changes in host innate and adaptive immunity and to examine potential mechanisms for putative immunosuppression, rapid *post-mortem* spleen and lung tissue harvest was performed at the bedsides of 40 patients who died in ICU with active severe sepsis to characterize their immune status at the time of death. Control spleens were obtained from patients who were declared brain-dead or had emergency splenectomy due to trauma; control lungs were obtained from transplant donors or lung cancer resections. Cytokine secretion assays and immunophenotyping of cell surface receptor-ligand expression profiles were performed to identify potential mechanisms of immune dysfunction. Immunohistochemical staining was performed to evaluate the loss of immune effector cells. Compared with controls, anti-CD3/anti-CD28–stimulated splenocytes from sepsis patients had significant reductions in cytokine secretion at 5 hrs: tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, and IL-10 ($p < 0.001$ for all). To assess the possible etiology for the markedly depressed cytokine secretion, the authors performed flow cytometric analysis and examined the expression of cell surface receptors important in cellular activation. The present study shows that splenocytes from sepsis patients had highly significant functional impairments, as evidenced by major reductions in cytokine secretion. Cytokine secretion in sepsis patients was generally less than 10% than in controls, independent of age, duration of sepsis, corticosteroid use, and nutritional status. Although differences existed between the spleen and lung, flow cytometric analysis showed increased expression of selected inhibitory receptors and ligands and expansion of suppressor cell populations in both organs. In the spleen, regulatory T cells (Treg) were increased approximately 2-fold in sepsis vs. control patients. In contrast, in the lung, no increase in Treg was detected, but there were increased cells consistent with a myeloid-derived suppressor cells (MDSC) phenotype. Expansion of suppressive cells, including Treg and MDSCs, has been reported in sepsis and provides another plausible mechanism for immunosuppression [5]. Unique differences in cellular inhibitory molecule expression existed in immune cells isolated from the lungs of sepsis patients vs. cancer patients and transplant donors. Antigen-presenting cells, i.e., dendritic cells and macrophages/monocytes, as well as tissue-specific macrophages, showed an immunosuppressive phenotype in sepsis as evidenced by decreased expression of CD86 and Human Leukocyte Antigen (HLA) – DR isotype. Immunohistochemical staining showed extensive depletion of splenic $CD4^+$, $CD8^+$, and HLA-DR-expressing cells and expression of ligands for inhibitory receptors on lung epithelial cells [4]. Evaluation of spleen tissue demonstrated a cellular loss in the periarteriolar lymphoid sheath – PALS and diminished number and size of splenic follicles in sepsis pa-

tients, as previously reported [4, 6]. These patients presented foci of bacterial infections that prolonged despite antimicrobial therapy, as well as a reactivation of latent viral infections [7, 8]. For a better understanding of the complex immune response in critically ill or injured patients with secondary sepsis, numerous pro- and anti-inflammatory mediators have been investigated, often with contradictory results. The impact of the type of bacterial causative agent on critically ill patients' immune response remains the subject of investigation. A relationship between the immune response and the survival of this patient population is still being investigated. A better insight into the immune response of critically ill patients might be gained by measuring the serum level of a larger number of inflammation mediators with predominantly pro-inflammatory features [IL-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, TNFα, IFN-γ, IFN-γ-inducible protein-10 (IP-10), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and MIP-1β] or anti-inflammatory features (IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33). Nowadays, sepsis is considered a dynamic syndrome characterized by many, often antagonistic phenomena, from hyperinflammation to anergy and immunoparalysis. The former concept of a pro-inflammatory process followed by a compensatory anti-inflammatory phase does not represent a common clinical pattern. These two processes more often progress with a significant degree of synchronization, though not necessarily at the same time. The hyperinflammatory phase, known as 'cytokine storm', is characterized by an uncontrollable production of pro-inflammatory mediators that often lead to organ damage/injury and bring about multiple organ dysfunction syndrome. Such a clinical scenario may lead to a premature death within a few days, and it may occur in the case of severe acute pancreatitis. The late stage of sepsis is dominated by a state of prolonged exhaustion of the immune effector cells, which results in immunosuppression [9, 10]. A delayed death from sepsis occurs either because of progressive exhaustion of the immune cells, resulting in secondary infections, or due to inflammation-induced organ damage/injury; in addition, there is often a combination of immunosuppression and persistent inflammation. The general problem with the investigation of sepsis in critically ill and injured patients is the heterogeneity of these patients, as is the case with the immune response. Over time, this response changes and is different in various patients with sepsis syndrome. Apart from these inter-individual differences, there are also significant intra-individual ones. The immune response of a patient is influenced by many variable factors, such as the time passed from the onset of the infection until the clinical manifestation of the disease, the source of infection, pathogen virulence, a possible former immunocompromisation of the patient, or gene-determined proclivity for a certain type of the immune response [11–13]. Of particular influence on the immune response is the patient's age. The population of the critically ill is getting older, and the immune response of older patients to insult is weakened, which is called immunosenescence [14]. The immune response of this patient population is also impacted by applied therapeutic measures, including medications and dysfunctional organism-supporting measures, such as various modes of hemodialysis and mechanical pulmonary ventilation. Bearing that in mind, the use of catecholamines, inotropes, and vasopressors has a great influence on the immune response [15]. Investigations have shown that the profile of cytokines in the critically ill with sepsis is impacted by a type of bacterial causative agent [16–18]. It is important to monitor the immune response for a longer period because of all of the mentioned factors that impact it and its variability. The aim of the study was to determine, according to the levels of pro-inflammatory (IL-1α, IL-1β, IL-6, IL-8, IL-12 p70, IL-17A, TNF-α, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β) and anti-inflammatory (IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33) mediators in the critically ill with secondary sepsis, whether the cytokine profile differs from the type of the bacterial causative agent, as well as to determine the influence of the cytokine profile on the outcome in this patient population. The outcome measure has been in-hospital mortality.

Methods

Patients

A total of 125 critically ill patients with secondary sepsis due to peritonitis, pancreatitis, and severe trauma, admitted to the Surgical ICU, were enrolled in a prospective study conducted in a tertiary university hospital (Military Medical Academy, Belgrade, Serbia). The study was carried out from November 2017 until October 2020 for a total duration of two years and eleven months. During the investigation period, the study encompassed critically ill patients, with ages ranging from 18 to 89 years. The average age was 57.7 ± 17.3 years. Of the total number of patients, 67.2% were males, and 32.8% were females. In concordance with the Declaration of Helsinki, the approval was obtained from the local Ethics Committee (date of issue November 29, 2017). In addition, informed consent was obtained from the patients or first-degree relatives. The study was conducted in accordance with the approved guidelines. The patients with secondary sepsis (underlying conditions being peritonitis, pancreatitis, and trauma) were enrolled if they had fulfilled current Sepsis-3 diagnostic criteria for sepsis (formerly severe sepsis) and/or septic shock [acute change in total Sequential Organ Failure Assessment (SOFA) score > 2 points, with vasopressors required to maintain mean arterial pressure (MAP) > 65 mm Hg, and serum lactate level > 2 mmol/L despite adequate volume resuscitation] [19]. The diagnostic criteria encompass any of the following variables thought to be a result of the infection: sepsis-induced hypotension, serum lactate levels greater than 2 mmol/L, urine output less than 0.5 mL/kg/hrs for more than 2 hrs despite adequate fluid resuscitation, acute lung injury with PaO_2/FiO_2 less than 250, creatinine greater than 2.0 mg/dL (34.2 micromol/L), platelet count less than 100,000, and coagulopathy with international normalized ratio – INR greater than 1.5. Moreover, critically ill patients with severe trauma (Injury Severity Score – ISS, determined using Abbreviated Injury Scale – AIS > 25 points), were enrolled after they developed secondary sepsis.

The exclusion criteria were as follows: secondary sepsis and/or septic shock with an underlying condition other than severe peritonitis, pancreatitis or trauma, malignant disease of any origin, long-term Surgical ICU stay before criteria, and a fulminant and pre-existing immunodeficiency. Out of 150 patients initially considered for enrolment, 25 were excluded.

Sampling and analysis

The vein blood samples were taken from 125 adult and critically ill patients with confirmed severe secondary sepsis as a complication of peritonitis, pancreatitis, or trauma once they fulfilled the criteria for a diagnosis of severe sepsis or septic shock (the first sample), which was repeated on the third and fifth day. The serum was extracted from the vein blood samples using a centrifuge at 1,000 revolutions for 10 min. The serum samples were frozen at -20 °C and then at -80 °C and kept until biomarker levels were determined. After the defrosting process, the biomarker levels in the serum samples were determined using the commercial flow cytometric kit (18-Plex Multiplex) using the flow cytofluorometry device (Beckman Coulter FC500). By doing so, the levels of pro-inflammatory (IL-1α, IL-1β, IL-6, IL-8, IL-12p70, IL-17A, TNF-α, IFN-γ, IP-10, MCP-1, MIP-1α, and MIP-1β) and anti-inflammatory (IL-4, IL-10, IL-13, IL-27, IL-31, and IL-33) mediators were determined at the three predefined time intervals. Detection sensitivity levels for cytokines (limit of detection – LOD) according to the manufacturer's note are the following: IL-1α < 2 pg/mL, IL-1β < 5 pg/mg, IL-6 < 5 pg/mL, IL-8 < 1 pg/mL, IL-12p70 < 3 pg/mL, IL-17A < 1 pg/mL, TNFα < 1 pg/mL, IFN-γ < 3 pg/mL, IP-10 < 3 pg/mL, MCP-1 < 2 pg/mL, MIP-1α < 2 pg/mL, MIP-1β < 5 pg/mL, IL-4 < 1 pg/mL, IL-10 < 2 pg/mL, IL-13 < 5 pg/mL, IL-27 < 5 pg/mL, IL-31 < 5 pg/mL and IL-33 < 5 pg/mL. Simultaneously, blood samples were also collected for blood culture. The type of bacterial causative agent was discovered through standard microbiological analyses. All the patients admitted to the ICU were being treated according to the latest guidelines for sepsis and septic shock treatment [20], along with adequate use of antibiotic therapy, vasoactive support, circulatory volume resuscitation, respiratory support through the application of various modes of mechanical ventilation, infection source surveillance, as well as by application of surgical treatment if deemed necessary. The outcome measure was in-hospital mortality; patients were monitored until hospital discharge (survivors) or in-hospital death (non-survivors).

Statistical analysis

Descriptive statistical methods and statistical hypotheses testing methods have been applied for the data analysis. The descriptive statistical methods included continuous variables shown as an arithmetic mean and standard deviation or a median and interquartile range (Q_1–Q_3) depending on distribution normality tested using the Shapiro-Wilk test. Frequency distributions of categorized variables are shown as absolute and relative numbers. The investigation of the hypothesis on the different significance of mean values of numerical characteristics involved the application of the Kruskal-Wallis test for independent samples, the Mann-Whitney *U* test for the test for sums of ranges, and the Friedman test and Wilcoxon test for investigating dependent samples. Chi-square and Fisher tests of precise probability were used to investigate frequency differences of categorized variables. The predictive power of all cytokines was tested by Receiver Operating Characteristic (ROC) analyses. The Area Under the ROC Curve (AUC) and 95% confidence intervals (CI), cut-off value with optimal sensitivity and specificity, and Youden index have all been calculated. Statistical hypotheses have been tested at the level of statistical significance (alpha level) of 0.05. All the analyses have been done by experts for medical statistics using the software program SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA).

Results

During the investigation period (two years and eleven months), the study encompassed 125 critically ill patients, with ages ranging from 18 to 89 years. The average age was 57.7 ± 17.3 years. Of the total number of patients, 84 (67.2%) were males, and 41 (32.8%) were females. Male gender was more common; the statistical difference was highly significant ($p < 0.001$); female patients were significantly older. According to blood culture, there were 28 (22.4%) patients with isolated Gram-positive pathogens, 29 (23.2%) of them with Gram-negative pathogens, 20 (16.0%) with polymicrobial blood culture, and 48 (38.4%) patients with negative blood culture. The overall in-hospital mortality amounted to 36.8%, and 79 (63.2%) patients survived. Demographic characteristics of the patients are shown in Table 1. Concerning the findings of the isolated blood culture, the comparison of mean cytokine values did not reveal a statistically significant difference between groups on the first day of measurement. When the mean cytokine values were compared to the nature of bacteremia on the third day of measurement, significant differences were detected between the groups for pro-inflammatory cytokines IL-1α ($p = 0.010$), IL-1β ($p = 0.009$), IL-6 ($p = 0.004$), IL-12p70 ($p = 0.011$), IL-17A ($p = 0.007$), TNF-α ($p = 0.006$), IFN-γ ($p = 0.018$), IP-10 ($p = 0.031$), MIP-1α ($p = 0.002$), MIP-1β ($p = 0.003$), MCP-1 ($p = 0.004$) and anti-inflammatory cytokines IL-4 ($p = 0.021$), IL-10 ($p = 0.008$), IL-13 ($p = 0.007$), IL-27 ($p = 0.005$), IL-31 ($p = 0.029$), IL-33 ($p = 0.006$). On the third day of measurement, mean cytokine values were statistically significantly lower in the polymicrobial group, as compared with the cytokine levels in the other investigated groups. Tables 2 and 3 present the third day of measurement's comparison of cytokines with regard to the blood culture findings. The general difference significance of the measured cytokines was tested further in order to ascertain among which blood cultures there is a difference in significance of the examined cytokine values. On the third day of measurement, statistically significantly lower values were obtained for the polymicrobial blood culture than for the

Table 1

Demographic characteristics of patients

Parameters	Values
Patients, n	125
Age (years), mean ± SD; med (min-max)	57.7 ± 17.3; 61 (18–89)
Gender, n (%)	
male	84 (67.2)
female	41 (32.8)
Sepsis-complicated primary condition, n (%)	
peritonitis	51 (40.8)
pancreatitis	33 (26.4)
trauma	41 (32.8)
Blood cultures, n (%)	
Gram-positive	28 (22.4)
Gram-negative	29 (23.2)
polymicrobial	20 (16.0)
negative	48 (38.4)
Outcome, n (%)	
non-survivors	46 (36.8)
survivors	79 (63.2)
Hospitalization length (days), mean ± SD; med (IQR)	29.9 ± 34; 22 (1–305)

n – number of patients; SD – standard deviation; med – median; min – minimum; max – maximum; IQR – interquartile range.

Table 2

Comparison of cytokines according to blood culture findings on the third day of measurement

Cytokines pg /mL	Blood cultures				*p*-value
	G+	G-	P	N	
IL-1α	119.5 (43.4–364.0)	128.7 (71.7–504.8)	62.0 (8.5–146.3)	266.1 (97.4–500.5)	0.010*
IL-1β	258.4 (134.6–445.8)	308.8 (84.4–547.8)	80.6 (0.6–222.7)	372.0 (181.3–477.0)	0.009*
IL-4	127.6 (43.6–253.8)	80.2 (39.0–283.0)	37.7 (0.0–92.9)	186.6 (54.8–333.1)	0.021*
IL-6	458.8 (247.9–857.6)	585.2 (352.9–1020.3)	173.8 (63.1–496.1)	658.1 (374.6–1098.1)	0.004*
IL-8	186.8 (73.8–576.8)	208.8 (81.2–461.1)	109.9 (46.0–287.4)	287.8 (132.9–660.7)	0.065
IL-10	46.4 (13.7–144.3)	30.8 (9.2–98.5)	14.4 (0.0–29.4)	73.2 (20.0–156.3)	0.008*
IL-12p70	66.2 (23.1–147.2)	78.6 (35.5–155.7)	31.3 (0.0–65.1)	112.2 (41.0–182.5)	0.011*
IL-13	214.0 (46.6–647.3)	173.2 (61.8–650.6)	42.0 (0.0–108.7)	416.4 (78.9–659.0)	0.007*
IL-17A	96.2 (37.2–226.2)	69.4 (43.0–302.8)	29.2 (0.9–56.7)	140.0 (48.3–300.8)	0.007*
IL-27	146.0 (49.1–430.4)	146.2 (73.6–322.8)	43.4 (4.4–100.4)	226.9 (75.8–495.8)	0.005*
IL-31	270.8 (125.8–560.0)	285.3 (154.6–542.6)	171.2 (46.4–268.4)	345.2 (197.9–624.3)	0.029*
IL-33	195.1 (76.2–313.6)	172.0 (79.4–347.9)	64.4 (10.3–122.2)	218.6 (124.7–412.8)	0.006*
TNF-α	258.8 (82.0–691.0)	212.8 (79.0–441.2)	74.4 (0.0–158.0)	345.2 (140.4–559.3)	0.006*
IFN-γ	74.4 (41.0–137.8)	71.4 (40.7–175.9)	30.2 (0.0–71.0)	113.1 (48.9–199.8)	0.018*
IP-10	166.4 (21.5–339.3)	252.0 (37.0–288.6)	44.6 (13.0–268.2)	244.4 (65.4–524.4)	0.031*
MIP-1α	148.6 (49.9–462.4)	133.2 (72.8–852.4)	59.2 (20.1–103.3)	226.6 (112.5–1136.6)	0.002*
MIP-1β	263.6 (131.9–410.4)	281.2 (132.6–475.1)	116.6 (44.0–198.8)	323.8 (157.6–747.1)	0.003*
MCP-1	221.2 (70.9–547.8)	212.2 (72.7–710.5)	80.4 (4.2–161.8)	424.1 (147.6–897.4)	0.004*

IL – interleukin; TNF – tumor necrosis factor; IFN – interferon; IP-10 – IFN-γ-inducible protein 10; MIP – macrophage inflammatory protein; MCP – monocyte chemoattractant protein; G+ – Gram-positive blood cultures; G- – Gram-negative blood cultures; P – polymicrobial blood cultures; N – negative blood cultures.

Data are presented as median (interquartile range). Significant differences are marked by *($p < 0.05$); *p*-values were based on the Kruskal-Wallis test.

Gram-positive blood culture, and for pro-inflammatory (IL-1β, IL-6, IL-12p70, IL-17A, TNF-α, IFN-γ, MIP-1α, MIP-1β, MCP-1) and anti-inflammatory (IL-4, IL-10, IL-13, IL-27, IL-33) cytokines. Statistically significantly lower values were obtained for the polymicrobial group than for the Gram-negative and negative ones for all of the investigated pro- and anti-inflammatory cytokines, except for IL-8 (Table 3). The only biomarker distinguished with statistically significantly higher values for the negative blood culture group than for the Gram-positive one was MIP-1α ($p = 0.048$). The examined cytokines' values have not significantly differed between the Gram-positive and Gram-negative blood culture groups, nor have they been statistically significantly different between the Gram-negative and negative ones. On the fifth day of measu-

Table 3

Levels of significance of statistical difference in mean cytokine values according to blood cultures on the third day of measurement

Cytokines pg/ mL	G+/P	G-/P	P/N
IL-1α	0.054	0.034*	0.001*
IL-1β	0.011*	0.019*	0.001*
IL-4	0.011*	0.045*	0.002*
IL-6	0.014*	0.004*	0.001*
IL-8	0.237	0.157	0.006*
IL-10	0.015*	0.048*	0.001*
IL-12p70	0.024*	0.014*	0.001*
IL-13	0.015*	0.016*	0.001*
IL-17A	0.008*	0.012*	0.001*
IL-27	0.017*	0.009*	0.001*
IL-31	0.081	0.050*	0.002*
IL-33	0.010*	0.016*	0.001*
TNF-α	0.009*	0.018*	0.001*
IFN-γ	0.026*	0.024*	0.003*
IP-10	0.262	0.047*	0.005*
MIP-1α	0.048*	0.016*	< 0.001*
MIP-1β	0.006*	0.005*	0.001*
MCP-1	0.021*	0.032*	< 0.001*

Data are presented as *p*-value significant; significant differences are marked by *($p < 0.05$); *p*-values were based on the Mann-Whitney *U* tests. For abbreviations, see Table 2.

Table 4

Comparison of cytokine values at three time intervals of measurement in patients with negative blood culture

Cytokines	Time intervals of measurement			*p*	*p*-value among measurements		
pg/mL	1st day	3rd day	5th day		1st– 3rd	1st– 5th	3rd– 5th
IL-1α	137.6 (59.1–313.0)	266.1 (97.4–500.5)	287.6 (69.9–698.6)	0.050*	0.002*	0.003*	0.452
IL-1β	307.7 (85.3–533.5)	372.0 (181.3–477.0)	365.8 (126.8–527.8)	0.297	0.194	0.093	0.823
IL-4	128.9 (37.4–232.0)	186.6 (54.8–333.1)	213.1 (63.0–383.9)	0.034*	0.003*	0.006*	0.482
IL-6	617.3 (254.6–1312.8)	658.1 (374.6–1098.1)	749.2 (304.2–1133.9)	0.102	0.034*	0.309	0.933
IL-8	211.0 (84.7–494.3)	287.8 (132.9–660.7)	301.8 (134.6–747.8)	0.061	0.030*	0.041*	0.648
IL-10	40.7 (14.4–85.7)	73.2 (20.0–156.3)	74.4 (15.4–226.8)	0.023*	0.003*	0.022*	0.802
IL-12p70	79.7 (30.3–128.7)	112.2 (41.0–182.5)	106.9 (45.0–273.8)	0.040*	0.001*	0.005*	0.436
IL-13	246.9 (42.2–468.8)	416.4 (78.9–659.0)	475.6 (80.9–961.1)	0.007*	0.001*	0.003*	0.354
IL-17A	72.5 (33.1–208.3)	140.0 (48.3–300.8)	195.4 (37.9–381.0)	0.006*	0.010*	0.003*	0.347
IL-27	159.5 (51.9–299.6)	226.9 (75.8–495.8)	287.4 (68.4–726.9)	0.054	0.002*	0.006*	0.677
IL-31	272.7 (152.4–477.4)	345.2 (197.9–624.3)	405.0 (202.6–704.7)	0.004*	0.005*	0.001*	0.426
IL-33	201.7 (65.9–291.7)	218.6 (124.7–412.8)	308.1 (102.4–418.4)	0.125	0.004*	0.028*	0.707
TNF-α	252.4 (107.6–448.9)	345.2 (140.4–559.3)	422.7 (114.7–825.1)	0.138	0.020*	0.029*	0.350
IFN-γ	77.9 (31.8–136.3)	113.1 (48.9–199.8)	122.1 (52.2–308.4)	0.187	0.002*	0.023*	0.861
IP-10	174.6 (46.2–256.3)	244.4 (65.4–524.4)	307.8 (100.0–737.4)	0.031*	0.039*	0.021*	0.712
MIP-1α	146.7 (58.6–305.0)	226.6 (112.5–1136.6)	510.6 (80.4–1148.0)	0.005*	0.001*	0.004*	0.667
MIP-1β	233.4 (137.3–423.8)	323.8 (157.6–747.1)	429.1 (182.8–600.4)	0.003*	0.001*	0.010*	0.563
MCP-1	263.6 (111.0–594.0)	424.1 (147.6–897.4)	601.6 (122.6–909.0)	0.099	0.005*	0.019*	1.00

Data are presented as median (interquartile range). Significant differences are marked by *($p < 0.05$). For abbreviations, see Table 2.

rement, the cytokines did not statistically significantly differ according to the isolated blood culture (Table 4). An absence of a statistically significant difference was ascertained at the three measurement intervals in patients with Gram-positive blood culture, except for MCP-1. The patients with Gram-positive blood culture present median values that are statistically significantly different at the monitored intervals for MCP-1 ($p = 0.047$), and they were statistically significantly higher on the third measurement day than on the first day, whereas the difference was not statistically significant between other intervals. None of the cytokines compared at the three time intervals in the patients with Gram-negative and polymicrobial blood cultures has presented a statistically significant difference. The cytokine values comparison at the

three measurement intervals in the patients with negative blood culture has shown the presence of a statistically significant difference for pro-inflammatory [IL-1α (p = 0.050), IL-12p70 (p = 0.040), IL-17A (p = 0.006), IP-10 (p = 0.031), MIP-1α (p = 0.005), MIP-1β (p = 0.003)] and anti-inflammatory [IL-4 (p = 0.034), IL-10 (p = 0.023), IL-13 (p = 0.007), IL-31 (p = 0.004)] cytokines. The patients with negative blood cultures presented statistically significantly higher values of the mentioned cytokines on the third and fifth day of measurement, compared to the first day, whereas the difference was not statistically significant between the third and the fifth day of measurement. Table 4 shows the cytokine values comparison at the three time intervals of measurement in the patients with negative blood cultures. At the first two time intervals of measurement, the statistical analysis determined no significant difference by comparing mean values of cytokines according to the outcome (survivor, non-survivor). The statistically significant difference appears only on the fifth day. At that time interval of measurement, the cytokine values were statistically significantly higher in survivors than in non-survivors – IL-1α, IL-1β, IL-8, IL-12p70, IL-17A, IFN-γ, IP 10, MIP-1α, MIP-1β, IL-4, IL-13, IL-27, IL-31, IL-33 (Table 5). The values of the investigated cytokines in non-survivors did not have statistically significant differences at the three time intervals. The comparison of the cytokine values in survivors at the three time intervals showed significant differences in biomarkers given in Table 6. The general tendency was that the levels of biomarkers increased at the monitored time intervals of measurement from the first until the fifth day. On the first day of measurement in the patients with secondary sepsis as a complication of peritonitis, the only important predictor of the fatal outcome was IL-17A, AUC of 0.665 (95% CI 0.519–0.791; p = 0.034). The levels of IL-17A on the first day of measurement, lower than the cut-off values (43.20 pg/mL), were moderate predictors of the fatal outcome in this group of patients (Figure 1). All the examined biomarkers on the third day of measurement became significant predictors of the polymicrobial blood culture. On the third day of measurement, the values of AUC for most cytokines were 0.7–0.8, which makes them quite discriminatorily powerful. The most powerful predictor of the polymicrobial blood culture was MIP-1β, AUC amounting to 0.772 (95% CI 0.684–0.845; p < 0.001). The MIP-1β levels lower than

Table 5

Cytokine values on the fifth day of measurement against the outcome

Cytokines pg/ mL	Outcome		*p*-value
	non-survivors	survivors	
IL-1α	89.5 (36.1–300.2)	352.9 (67.0–697.7)	0.025*
IL-1β	176.4 (69.0–375.2)	382.7 (99.5–714.5)	0.028*
IL-4	101.8 (0.0–217.4)	207.0 (47.2–443.6)	0.043*
IL-6	536.1 (164.5–1138.7)	693.8 (249.6–1020.3)	0.684
IL-8	124.6 (55.5–488.5)	403.0 (108.4–846.8)	0.021*
IL-10	30.3 (0.0–124.2)	65.8 (13.0–65.8)	0.181
IL-12p70	45.8 (2.8–109.1)	112.9 (38.9–379.7)	0.012*
IL-13	89.0 (7.6–526.7)	475.6 (52.6–990.3)	0.032*
IL-17A	51.6 (13.6–238.3)	195.4 (37.1–450.8)	0.017*
IL-27	89.0 (13.0–322.1)	277.8 (62.7–676.3)	0.022*
IL-31	154.2 (83.8–487.5)	471.0 (226.0–904.1)	0.002*
IL-33	103.2 (28.9–350.6)	301.2 (80.2–531.4)	0.012*
TNF-α	154.2 (24.2–523.9)	422.7 (75.4–860.0)	0.055
IFN-γ	58.8 (10.9–121.0)	122.2 (33.0–257.8)	0.038*
IP-10	88.4 (19.5–490.6)	332.9 (96.6–717.1)	0.014*
MIP-1α	98.2 (50.8–554.8)	237.0 (76.4–1127.8)	0.042*
MIP-1β	151.4 (97.5–439.9)	446.4 (159.1–637.3)	0.026*
MCP-1	159.6 (67.3–735.0)	501.3 (83.4–884.5)	0.325

Data are presented as median (interquartile range). Significant differences are marked by *(p < 0.05).
For abbreviations, see Table 2.

Table 6

Comparison of cytokine values in survivors at three time intervals of measurement

Cytokines pg/mL	Time intervals of measurement			*p*	*p*-value among measurements		
	1st day	3rd day	5th day		1st–3rd	1st–5th	3rd–5th
IL-1α	126.4 (59.1–348.0)	138.4 (75.0–465.8)	352.9 (67.0–697.7)	0.036*	0.057	< 0.001*	0.290
IL-17A	69.7 (35.6–257.0)	97.9 (40.6–271.1)	195.4 (37.1–450.8)	0.019*	0.156	0.003*	0.050*
IL-31	268.2 (161.2–551.0)	305.3 (178.0–566.8)	471.0 (226.0–904.1)	0.007*	0.091	0.001*	0.088
IP-10	175.8 (48.8–359.7)	195.5 (42.7–507.4)	332.9 (96.6–717.1)	0.004*	0.231	0.002*	0.138
MIP-1α	120.3 (60.2–353.1)	176.3 (73.6–847.6)	237.0 (76.4–1127.8)	0.044*	0.006*	0.003*	0.726
MIP-1β	227.8 (116.3–471.8)	291.0 (129.1–517.0)	446.4 (159.1–637.3)	0.028*	0.030*	0.015*	0.869

Data are presented as median (interquartile range). Significant differences are marked by *(p < 0.05).
For other abbreviations, see Table 2.

the cut-off values (215.4 pg/mL) were good predictors of the polymicrobial blood culture. The AUC values for cytokines as predictors of polymicrobial blood culture on the third day of measurement are given in Table 7. On the third day of measurement, all the biomarkers became significant predictors of the negative blood culture. The values of AUC for these biomarkers were in the range of 0.6–0.7. The most powerful predictor of the negative blood culture was MIP-1α, whose levels, higher than the cut-off values (176.3 pg/mL), were good predictors of the negative blood culture. The AUC values for cytokines as predictors of negative blood culture on the third day of measurement are given in Table 8. On the third day of measurement, AUC analysis did not reveal statistical significance in the prediction of either the Gram-positive or Gram-negative blood cultures. As for the patients with Gram-positive, polymicrobial, and negative blood cultures, the third day of measurement revealed that cytokines were statistically insignificant for discrimination between the non-survivors and survivors. As for the patients with Gram-negative blood cultures, all the biomarkers were statistically significant predictors of the fatal outcome, except for IL-6. The values of AUC for all other biomarkers on the third day of measurement were in the range of 0.7–0.8. The most powerful predictor for the fatal outcome became MIP-1α, AUC amounting to 0.796. The values of the MIP-1α on the third day of measurement, less than the cut-off values

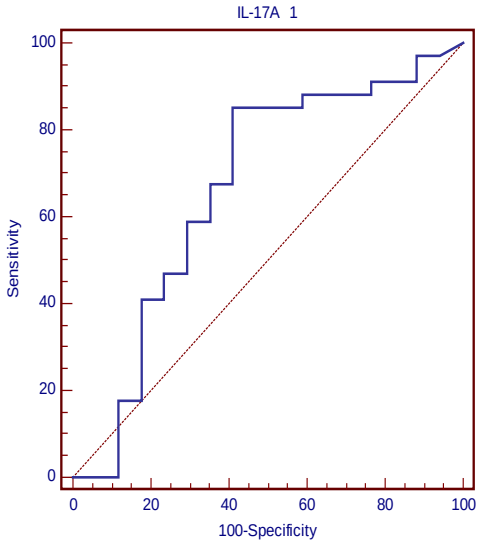

Fig. 1 – Receiver operating characteristic curve for interleukin (IL)-17A in prediction of peritonitis-related fatal outcome on the first day of measurement.

Table 7

The AUC values for cytokines as predictors of polymicrobial blood cultures on the third day of measurement

Cytokines pg/mL	AUC	*p*-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %	Youden index
			lower bound	upper bound				
IL-1α	0.733	< 0.001*	0.642	0.811	82.7	68.7	72.7	0.41
IL-1β	0.752	< 0.001*	0.663	0.828	248.9	87.5	58.6	0.46
IL-4	0.730	< 0.001*	0.639	0.808	96.9	81.2	60.6	0.42
IL-6	0.760	< 0.001*	0.672	0.835	205.0	62.5	86.9	0.49
IL-8	0.669	0.011*	0.575	0.754	155.9	68.7	63.6	0.32
IL-10	0.734	< 0.001*	0.643	0.812	30.4	81.2	64.6	0.46
IL-12p70	0.739	< 0.001*	0.649	0.817	70.0	87.5	56.6	0.44
IL-13	0.752	< 0.001*	0.663	0.828	114.2	81.2	63.6	0.45
IL-17A	0.759	< 0.001*	0.670	0.834	65.1	87.5	59.6	0.47
IL-27	0.757	< 0.001*	0.668	0.832	104.2	81.2	68.7	0.50
IL-31	0.711	< 0.001*	0.620	0.792	200.3	75.0	71.7	0.47
IL-33	0.755	< 0.001*	0.666	0.831	165.9	87.5	61.6	0.49
TNF-α	0.754	< 0.001*	0.665	0.830	159.6	81.2	67.7	0.49
IFN-γ	0.727	< 0.001*	0.636	0.806	59.3	75.0	67.7	0.43
IP-10	0.684	0.004*	0.591	0.768	68.9	68.7	71.7	0.40
MIP-1α	0.751	< 0.001*	0.661	0.827	110.5	81.2	70.7	0.52
MIP-1β	0.772	< 0.001*	0.684	0.845	215.4	87.5	65.7	0.53
MCP-1	0.748	< 0.001*	0.658	0.824	167.5	81.2	63.6	0.45

AUC – Area Under ROC Curve; ROC – Receiver Operating Characteristic. For other abbreviations, see Table 2. Significant differences are marked by *($p < 0.05$).

Table 8

The AUC values for cytokines as predictors of negative blood cultures on the third day of measurement

Cytokines pg/mL	AUC	*p*-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %	Youden index
			lower bound	upper bound				
IL-1α	0.635	0.014*	0.540	0.722	189.2	65.1	66.7	0.32
IL-1β	0.610	0.046*	0.515	0.700	292.4	60.5	62.5	0.23
IL-4	0.605	0.057	0.510	0.695	133.0	62.8	62.5	0.25
IL-6	0.626	0.022*	0.531	0.714	350.8	81.4	41.7	0.23
IL-8	0.622	0.026*	0.527	0.711	233.8	62.8	59.7	0.23
IL-10	0.632	0.016*	0.537	0.720	61.4	58.1	68.1	0.26
IL-12p70	0.620	0.029*	0.525	0.709	82.4	65.1	63.9	0.29
IL-13	0.623	0.025*	0.528	0.711	257.4	67.4	63.9	0.31
IL-17A	0.610	0.046*	0.515	0.700	97.9	58.1	62.5	0.21
IL-27	0.633	0.015*	0.538	0.721	169.1	67.4	65.3	0.33
IL-31	0.615	0.038*	0.519	0.704	163.4	90.7	31.9	0.23
IL-33	0.627	0.020*	0.532	0.716	123.1	76.7	47.2	0.24
TNF-α	0.616	0.036*	0.520	0.705	221.7	69.8	56.9	0.27
IFN-γ	0.618	0.032*	0.523	0.707	100.6	62.8	66.7	0.30
IP-10	0.614	0.039*	0.518	0.703	34.1	88.4	33.3	0.22
MIP-1α	0.669	0.002*	0.576	0.754	176.3	69.8	65.3	0.35
MIP-1β	0.631	0.017*	0.536	0.719	397.0	48.8	79.2	0.28
MCP-1	0.649	0.006*	0.555	0.736	116.9	81.4	45.8	0.27

Significant differences are marked by *($p < 0.05$).
For abbreviations, see Tables 2 and 7.

Table 9

Fatal outcome prediction on the third day of measurement of cytokines in patients with Gram-negative blood culture

Cytokines pg/mL	AUC	*p*-value	95% confidence interval		Cut-off value	Sensitivity %	Specificity %	Youden index
			lower bound	upper bound				
IL-1α	0.782	0.002*	0.587	0.914	102.0	71.4	85.7	0.57
IL-1β	0.776	0.003*	0.579	0.910	241.5	66.7	85.7	0.52
IL-4	0.762	0.006*	0.564	0.901	60.7	76.2	71.4	0.48
IL-6	0.551	0.694	0.353	0.738	1020.3	85.7	42.9	0.29
IL-8	0.728	0.026*	0.528	0.877	114.0	81.0	71.4	0.52
IL-10	0.748	0.012*	0.549	0.891	28.3	71.4	85.7	0.57
IL-12p70	0.776	0.003*	0.579	0.910	68.5	66.7	85.7	0.52
IL-13	0.782	0.002*	0.587	0.914	89.7	71.4	85.7	0.57
IL-17A	0.772	0.004*	0.575	0.908	60.4	66.7	85.7	0.52
IL-27	0.755	0.009*	0.557	0.896	107.3	76.2	71.4	0.48
IL-31	0.762	0.006*	0.564	0.901	183.2	85.7	71.4	0.57
IL-33	0.789	0.001*	0.594	0.919	123.1	71.4	85.7	0.57
TNF-α	0.755	0.009*	0.557	0.896	139.6	76.2	71.4	0.48
IFN-γ	0.776	0.003*	0.579	0.910	61.2	66.7	85.7	0.52
IP-10	0.762	0.006*	0.564	0.901	30.4	90.5	57.1	0.48
MIP-1α	0.796	< 0.001*	0.602	0.923	79.0	85.7	71.4	0.57
MIP-1β	0.755	0.009*	0.557	0.896	215.0	81.0	71.4	0.52
MCP-1	0.759	0.007*	0.560	0.898	111.8	71.4	85.7	0.57

Significant differences are marked by *($p < 0.05$).
For abbreviations, see Tables 2 and 7.

(79.0 pg/mL), were good predictors of the fatal outcome in the patients with Gram-negative blood culture. The prediction of the fatal outcome on the third day of measurement of cytokines in the patients with Gram-negative blood culture is given in Table 9.

Discussion

The progress in the therapeutic measures of support for organ systems in the critically ill with sepsis and/or trauma has led to improvements in their thirty-day survival rate [21]. That has shown changes in the immunoinflammatory response in this category of patients. In the past, the pro-inflammatory response was considered a generator for premature mortality (for the first couple of days), and the compensatory anti-inflammatory response would induce organ damages/injuries, immunosuppression, and mortality after a few weeks [22, 23]. New observations have revealed that the long-term and simultaneous pro-inflammatory and anti-inflammatory response, the heart of which is a dysfunctional,

innate, and suppressed immunity, culminates in persistent organ damage/injury and fatal outcome [24, 25]. Immune dysfunction has a significant role in the delayed, late death of a critically ill patient. Regarding the described immune response in the critically ill with sepsis, there are various data in the literature – that is why this represents a topical focus of research. The importance of the issue is found in the fact that the new definition of sepsis (Sepsis-3) from 2016 includes the term 'dysregulation of immune response' [20], which defines sepsis as a life-threatening dysfunction of organs caused by a dysregulation of the host's response to infection. This problem is complex because there is a subgroup of patients with sepsis dominated by the pro-inflammatory immune response and a larger number of patients in whom the dysregulation of immune response is manifested through immunosuppression. In order to discriminate these two categories of patients, it is necessary to perform monitoring of immune phenotypes for every single patient, which would help to reach a desired individual therapeutic approach to immunomodulation [26, 27]. So far, the role of the complex immune response in sepsis has not been fully clarified and represents a subject matter of research with often contradictory results. The mentioned 18 mediators in the previous investigations have not been evaluated simultaneously, and their mutual relationship in conditions of chronic critical disease is yet to be researched. Our prospective and observational study has focused on the simultaneous measurement of pro- and anti-inflammatory cytokines in well-defined populations of critically ill patients with severe secondary sepsis as a complication of peritonitis, pancreatitis, or trauma to minimize heterogeneous differences accentuated in sepsis. Simultaneous assessment of a larger number of cytokines in sepsis at different time intervals may identify complex cytokine patterns [28], which reflect the immune response of critically ill patients. The appearance of multiplex testing has made it possible to study a wider immuno-inflammatory response, and this new technique has been proposed as a potential diagnostic implement for sepsis, owing to its possibility to characterize specific subgroups of patients with sepsis [19]. The patterns of pro- and anti-inflammatory cytokines in patients with sepsis have been widely researched in previous studies [28–31]. Although the international guidelines for severe sepsis and septic shock treatment do not take into account the type of the pathogenic causative agent, *in vitro* data suggest that there are numerous differences in the cytokine profiles and mortality rates between subclasses, such as the Gram-negative bacteria (GNB) and Gram-positive bacteria (GPB) [32]. Several studies have demonstrated the significant influence of the type of bacterial causative agent on the cytokine profile in the critically ill patients [18, 33]. However, although there is a considerable percentage of microbiologically undocumented infections [34] in the literature available for our research, we have not found a study on critically ill patients in ICU that would report on the cytokine profile in the negative blood cultures. Only one study has had information on the cytokine measurement in the negative blood cultures in patients with sepsis in the emergency department [35]. The aim of this prospective observational study was to investigate whether the levels of the inflammatory mediators in plasma differ between the sepsis-affected patients with bacteremia and those without bacteremia during the preliminary hospitalization phase. In total, 80 patients were divided into two main subgroups, according to whether bacteremia could have been discovered. The samples of plasma in this study were collected within 24 hrs (mostly within 3 hrs) from the time of hospitalization, and they were measured only at that interval. The authors have come to the conclusion that bacteremia was related to the higher levels of the inflammatory mediators, as opposed to our results, which reveal that the inflammatory mediators were significantly lower in the polymicrobial blood culture compared to the Gram-negative, Gram-positive, or negative ones on the third day of measurement. At other measurement intervals, the cytokine profile differences were not significant regarding blood culture. The investigated cytokines have proven to be good predictors for the polymicrobial and negative blood cultures, whereas the discrimination of the Gram-negative and Gram-positive blood cultures has not produced any of the cytokines as a good predictor. Feezor et al. [36] have determined that considerably higher levels of pro-inflammatory cytokines in 52 patients with sepsis result from the GPB compared to the Gram-negative ones. Baseline levels of TNF-α, IL-1Ra, IL-8, IL-10, IL-18BP, procalcitonin, and protein C in plasma were not significantly different between septic patients with Gram-positive and Gram-negative infections. In contrast, plasma IL-1β, IL-6, and IL-18 concentrations were significantly higher among patients with sepsis due to GPB than patients with sepsis due to GNB despite no significant differences in the magnitude of the physiologic response (Acute Physiology And Chronic Health Evaluation – APACHE II score), the degree of organ injury (Multiple Organ Dysfunction Score), or other pro-inflammatory cytokines. These findings suggest that the patterns of plasma cytokine appearance may differ between patients with sepsis due to GNB and GPB [36]. On the other hand, researchers of another study have determined that the GNB induce more pro- and anti-inflammatory cytokines compared to the GPB [18]. The aim of their study was to determine whether the early cytokine profile can discriminate between GPB and GNB, respectively, in critically ill patients with severe abdominal sepsis. Blood samples were obtained from 165 adult patients with confirmed severe abdominal sepsis. Levels of the pro-inflammatory mediators TNF-α, IL-8, IL-12, and IFN-γ and the anti-inflammatory mediators IL-1Ra, IL-4, IL-10, and Transforming growth factor (TGF)-β1 were determined and correlated with the nature of the bacteria isolated from the blood culture. The cytokine profile in their study indicated that the TNF-α levels were 2-fold, IL-8 levels were 3.3-fold, IFN-γ were 13-fold, IL-1Ra were 1.05-fold, IL-4 were 1.4-fold, and IL-10 were 1.83-fold higher in the GNB group compared with the GPB group [18]. We have expected to find a larger difference in the immuno-inflammatory response between the Gram-negative and Gram-positive bacterial infections, but unlike the preceding studies [18, 36] significantly larger differences in the inflammatory mediators have not been detected. Mortality may be impacted by the type of an infec-

tious agent. Some authors have reported increased mortality with GPB, while other researchers report significantly higher mortality rates with GNB [37]. Zahar et al. [38] have not demonstrated a relationship between the bacteria and mortality. Our study's prediction of the fatal outcome has singled out only the Gram-negative blood culture, wherein all the cytokines, except for IL-6, were significant predictors for the fatal outcome, with AUC values in the range of 0.7–0.8 at the third measurement interval. The most powerful predictor for the fatal outcome became MIP-1α, with values lower than 79 pg/mL in non-survivors compared to survivors. MIP-1α is an inflammatory chemokine produced by cells during infection or inflammation. It belongs to the CC chemokine family, which displays potent chemotactic properties. This protein was called MIP-1α because of its biological function of inducing an inflammatory response characterized by neutrophil infiltration. It performs various functions, such as recruiting inflammatory cells, wound healing, inhibition of stem cells, and maintaining effector immune response [39]. Most mature hematopoietic cells can induce the synthesis of MIP-1α. Monocytes, T lymphocytes, B lymphocytes, neutrophils, dendritic cells, and natural killer cells are known to secrete MIP-1α. Under normal conditions, synthesis of MIP-1α occurs at very low levels. However, upon stimulation of receptive cells with endotoxins such as lipopolysaccharide or proinflammatory cytokines, cellular signaling events are activated, and this activation induces increased production of MIP-1α. Elevated circulating MIP-1α levels are also found in patients with septic shock [40]. Identification of critically ill patients with higher death risk is important [41]. The total in-hospital mortality in our research amounted to 36.8%. Afuwape et al. [42] have analyzed the influence of the cytokine response (TNF-α, IL-1α) on the survival of patients with generalized peritonitis for six months in a pilot study during which they concluded that the TNF-α lower levels and the IL-1α higher levels are related to survival. As the only significant predictor of fatal outcome on the first day of measurement, our study revealed IL-17A, with lower values in non-survivors compared to survivors in the case of secondary sepsis occurring as a complication of peritonitis. On the other hand, the levels of other cytokines correlated with the outcome only on the fifth day of measurement and were higher in the survivors compared to non-survivors. From the clinical point of view, the fifth day of measurement is quite late for the outcome prediction. The studies investigating the role of IL-17A on animal models of sepsis have published that IL-17A causes considerable pathology and that a significantly improved survival included the elimination of this cytokine [43]. Later studies have published opposite results [44], and the contemporary literature comprises numerous studies that demonstrate mixed blockade effects of IL-17A in sepsis [45]. In a study carried out on humans, done by Ahmed Ali et al. [46], the increased levels of IL-17A in the serum predict the development of sepsis and mortality in patients with polytrauma. On the first day of measurement, the patients with secondary sepsis as a complication of pancreatitis or trauma did not present any of the examined cytokines as having a discriminatory power with regard to the prediction of the fatal outcome. Despite the high prevalence of negative blood culture, previous studies have not reported on cytokine profiles in critically ill septic patients in the ICU. We have expected to find a much weaker production capacity of cytokines in a negative blood culture. These are some of the questions solicited by our research: Why would we expect a lot of cytokines where bacteria are low in number, and what is hiding in the negative blood cultures? What is exactly the cytokine production capacity like in negative sepsis? Although this study cannot answer all the questions, its findings offer insight into some of these possibilities. The assumed reasons include exposure to antibiotics prior to being admitted to the ICU, as well as a possible presence of slow-growing or fastidious bacteria. Molecular techniques based on the polymerization chain reaction (PCR) may improve discovery rates of pathogens, and many patients with clinical sepsis are truly PCR-positive but negative in blood culture [47]. Our present study has several limitations – this is an observational study carried out within one institution on a relatively small sample size (125 patients). That is why the tendencies and patterns discovered herein should be confirmed in a larger patient population through a multicentric study. We cannot use our results to generalize about other groups of critically ill and traumatized patients. The overall applicability of our results to other forms of sepsis is unclear. Our findings represent preliminary results, and further investigations are justified with a larger number of patients and other subpopulations of septic patients.

Conclusion

On the third day of measurement, we demonstrated statistically significant differences in cytokine values according to the type of bacteremia, with the lowest levels of cytokines in the polymicrobial blood culture. IL-17A is a good predictor of the outcome of secondary sepsis occurring as a complication of peritonitis. The low level of IL-17A in these patients predicts a fatal outcome. On the other hand, only on the fifth day of measurement did the levels of other cytokines correlate with the outcome, and they were higher in survivors compared to non-survivors.

Conflict of interest

The authors report no conflict of interest.

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The effect of different types of storage solutions on saphenous vein endothelial integrity in diabetic patients undergoing coronary artery bypass grafting

Uticaj različitih tipova prezervacionih rastvora na integritet endotela safenske vene u koronarnoj hirurgiji kod bolesnika sa dijabetesom melitusom podvrgnutih hirurškoj revaskularizaciji miokarda

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Abstract

Background/Aim. Taking into consideration the justified popularity of total arterial revascularization, the saphenous vein graft (SVG) is still one of the most utilized conduits in coronary artery bypass grafting (CABG). One of the determining factors of this conduit's durability is its endothelial integrity at the time of surgery. The aim of the study was to investigate the effect of different storage solutions on SVG endothelial integrity in patients with diabetes mellitus (DM) type 2 (T2DM) and non-T2DM patients undergoing CABG. The solutions under evaluation were heparinized saline solution (0.9% NaCl), heparinized autologous whole blood, Bretschneider (histidine-tryptophan-ketoglutarate – HTK) solution, and fresh frozen plasma (FFP) solution. **Methods.** Forty patients were included in this study and were divided into two groups: group A with 23 T2DM patients and group B with 17 non-T2DM patients. The effects of these solutions were examined by immunohistochemical staining with anti-CD34 antibodies and morphometric comparison in histologic samples of T2DM patients undergoing CABG between July 2021 and September 2022 with samples provided by non-T2DM patients. **Results.** In this study, the FFP solution showed the most prominent positive effect on the preservation of SVG endothelial integrity, with an average cell integrity preservation of 92.2%. HTK solution was found to be the least effective, with an endothelial cell preservation integrity of 26.77%. There was no marked statistically significant difference in results from groups A and B. There was a noticeable contrast in preserving SVG endothelial integrity between the two patient groups, T2DM and non-T2DM patients, although it was not statistically significant. **Conclusion**. The storage solution with the most beneficial effect on SVG endothelial integrity preservation was the FFP solution when harvested *via* the conventional open method in CABG.

Key words:
coronary artery bypass; diabetes mellitus, type 2; histological techniques; organ preservation solutions; saphenous vein; transplants.

Apstrakt

Uvod/Cilj. Uprkos opravdanom porastu popularnosti totalne arterijske revaskularizacije, graft safenske vene (GSV) ostaje jedan od najkorišćenijih provodnika u hirurškoj revaskularizaciji miokarda (*coronary artery bypass grafting* – CABG). Jedan od odlučujućih faktora trajnosti tog provodnika je integritet njegovog endotela u vreme operacije. Cilj rada bio je da se kod bolesnika podvrgnutih CABG sa tip 2 dijabetesom melitusom (T2DM) i kod bolesnika bez T2DM ispita uticaj prezervacionih rastvora na integritet endotelnih ćelija GSV. Rastvori koji su procenjivani bili su: heparinizirani fiziološki rastvor (*sol.* 0,9% NaCl), heparinizirana puna autologna krv, Bretschneider-ov histidin-triptofan-ketoglutarat (HTK) rastvor i rastvor sveže zamrznute plazme (SZP). **Metode**. U studiju je bilo uključeno 40 bolesnika podeljenih u dve grupe: grupu A, u kojoj su bila 23 bolesnika sa T2DM i grupu B, sa 17 bolesnika bez T2DM. Efekti prezervacionih rastvora ispitivani su imunohistohemijskim bojenjem anti-CD34 antitelom i morfometrijskim poređenjem histoloških uzoraka bolesnika sa T2DM, podvrgnutih CABG između jula 2021. i septembra 2022. godine, sa uzorcima koji su dali bolesnici koji nisu imali T2DM. **Rezultati**. Najbolji efekat

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na očuvanje integriteta endotela GSV imao je rastvor SZP, sa prosečnim očuvanjem integriteta endotela od 92,2%. Najnepovoljniji efekat imao je rastvor HTK, sa prosečnim očuvanjem integriteta endotela od 26,77%. Nije bilo statistički značajne razlike rezultata između grupa A i B. Postojao je primetan, mada ne statistički značajan, kontrast u očuvanju integriteta endotela GSV između bolesnika sa T2DM i bolesnika bez T2DM. **Zaključak.** U konvencionalnom metodu CABG, prezervacioni rastvor sa najboljim efektom na očuvanje integriteta endotela GSV bio je rastvor SZP.

Ključne reči:
aortokoronarno premošćavanje; dijabetes melitus, insulin-nezavisni; histološke tehnike; rastvori za čuvanje organa; v. saphena; graftovi.

Introduction

Atherosclerotic coronary artery disease is a leading cause of mortality in the population over 65 years of age worldwide. Risk factors, including diabetic disease, have been a significant contributor to mortality over the two decades of 1990–2010, especially in developing countries. On the contrary, high-income countries are showing a reduction in risk factors due to improved awareness [1].

Total arterial revascularization (TAR) has been gaining momentum worldwide [2]. All things considered, the saphenous vein (SV) graft (SVG) is still the most utilized conduit in coronary surgery, with coronary artery bypass graft (CABG) surgery being one of, if not the most performed type of surgery worldwide. If we consider that only 5–10% of CABG patients will receive TAR, the average number of conduits *per* patient being 3.1 [3], and with more than 500,000 patients every year undergoing CABG with at least one SVG, the number gets only bigger. SVG will stick around in coronary surgery for a long time due to its readiness, ease of harvesting and manipulation, as well as the possibility of creating different graft configurations.

SVG patency is, to this day, the main flaw of this conduit. SVGs are known for their high rate of occlusion (3–12% before hospital discharge, 8–25% in the first year postoperatively, and 50–60% ten years postoperatively) [4] regardless of the advance in surgical technique and pharmacological therapy. Approximately 13% of all CABG patients will be eligible for redo-surgery in the first ten years after the first CABG, while 18% of all percutaneous coronary intervention (PCI) procedures are performed on the CABG patients, and 6% of all PCI procedures are performed on SVGs, indicating the necessity for repeat revascularization [5]. Excluding the harvesting technique [6, 7] and revascularization strategy [8], one of the factors contributing to SVG longevity is the type of storage solution used during surgery. The choice of solution type has shown to have a wide spectrum of effects on the endothelial layer's integrity, both beneficial and disadvantageous [9].

Diabetes mellitus (DM) type 2 (T2DM), is an important cardiovascular risk factor, and the prevalence of T2DM in patients undergoing CABG surgery is nearly 30–40% [10].

The metabolic effects of T2DM alter the walls of blood vessels, beginning with the loss of proper endothelial function, oxidative stress, low-intensity inflammation, and high platelet adhesion rate [11]. Advanced glycation end products and their receptor activation may accelerate SVG smooth muscle cell proliferation in DM patients. Even with adequate glycemic control, the intimal hyperplasia is more pronounced in the SVG of DM patients when compared with patients not affected by this disease [10]. In a study published in 2008, graft occlusions were more common among diabetics compared with non-diabetics. DM was associated with lower vein graft patency but similar arterial graft patency in comparison to non-diabetics. Uncontrolled DM and long duration of disease were found to be significant predictors for graft occlusion [12]. The aim of this study was to determine the difference in SVG endothelial integrity between patients with T2DM and non-T2DM patients undergoing CABG.

Methods

Participants and randomization

Patients with isolated coronary artery disease and the use of at least one SVG were eligible to enroll. Additionally, patients were distributed into two groups according to the presence of T2DM. They were then distributed into four groups according to the type of storage solution used: heparinized saline (0.9% NaCl), heparinized whole autologous blood, fresh frozen plasma (FFP) solution, and Bretschneider's (histidine-tryptophan-ketoglutarate – HTK) solution. Patients with indications for valvular, aortic, redo, and peripheral artery surgery, as well as leg varicosities, postthrombotic syndrome, and state after thrombophlebitis of the veins of lower extremities were excluded from participating in this study. Color duplex scan examination of the great SV was performed preoperatively (Mindray DC-80A X-insight, Shenzhen, China). Randomization was conducted by the method of chance up until the number of required applicants was achieved. The research was conducted in the facilities of the Cardiac Surgery Clinic, the Emergency Center of the University Clinical Center Niš, Serbia, and the Department of Histology and Embryology Laboratory. The study was approved by the Emergency Center of the University Clinical Center Niš Ethics Committee (No 3830/7, from February 4, 2020) and the Faculty of Medicine in Niš Ethical Board (No. 12-15637-2/8, from December 24, 2019). A written informed consent was obtained from every participant in the study at the time of hospital admittance. The total number of patients considered was 200. The number of patients enrolled in this study was 40, all with atherosclerotic coronary disease and indicated for CABG surgery (Table 1).

Table 1

Comparison of T2DM vs. non-T2DM patients

Parameter	Patients	
	T2DM	non-T2DM
Gender		
male	18	13
female	5	4
Age (years)	66 (41–80)	68 (42–80)
EuroSCORE	3.48 (0.73–10.1)	3.39 (0.81–11.2)
BMI	28.3 (23.7–34.5)	26.1 (22.1–33.2)
BSA	2.1 (1.7–2.5)	1.9 (1.6–2.3)
LVEF (%)	44.3 (25–68)	46.1 (27–69)
Current smoker	9 (4 female, 5 male)	10 (4 female, 6 male)
Hypertension	19 (95) using anti-HTN drugs	18 (90) using anti-HTN drugs
HbA1c (%)	6.61 (5.2–8.8)	not measured

T2DM – type 2 diabetes mellitus; BMI – body mass index; BSA – body surface area; LVEF – left ventricular ejection fraction; HbA1c – glycated hemoglobin; HTN – histidine-tryptophan-ketoglutarate.
All values are expressed as numbers, mean (minimum-maximum), or numbers (percentages).

Surgical procedure

The harvesting and storage of the great SV was conducted by two experienced surgeons, both of whom perform more than 100 SVG harvesting procedures annually using the conventional open harvesting technique. The surrounding fat tissue was separated by blunt preparation and sharp dissection from the SV adventitial layer. The initial surgical cut was located 5 cm above the medial malleolus and continued cranially following the anatomic projection of the great SV. The length was determined preoperatively based on the number of planned bypasses. A plastic cannula was placed through the venotomy site and secured with a ligature, with side branches either ligated or clipsed. The SVG was then dilated and checked for leakage using one of the four storage solutions with the help of a 20 mL plastic syringe. The dilation pressure did not exceed 200 mmHg. After this procedure, a sample of the SVG 0.5–2 cm long proximal to the initial incision, 5 cm above the medial malleolus was obtained by sharp section and placed into an inox container (by submersion) filled with the designated storage solution already used for dilation at the room temperature (the operating theater temperature varies from 10–14 ˚C) for an average time period of 32.1 min (12–67 min). The sample was stored accordingly until the proximal anastomosis stage between the coronary artery and the SVG. During this time period, the SV specimen is in a warm ischemic period. The sample is then taken from the container with the solution for temporal simulation purposes and placed into a plastic container filled with 4% buffered water formaldehyde solution. This procedure is done in the setting of full heparinization according to the cardiopulmonary bypass protocol. Using 1 mL of unfractionated heparin containing 5,000 IU, 100 mL of heparinized 0.9% NaCl solution was prepared in an inox container. FFP solution was obtained from the Blood Transfusion Institute in Niš on a daily basis at the time of the patient's arrival at the Operating Room, thawed and ready for use. One unit contains 250 mL of plasma and 500 mg of fibrinogen in a citrate anticoagulant, with an estimated albumin content of 26 g/L and 57 g/L of protein. Using 1 mL of unfractionated heparin containing 5,000 IU Bretschneider's solution, 100 mL of autologous whole blood was obtained *via* the distal part of the central venous catheter placed in a usual manner and prepared in an inox container.

Histological and immunohistochemical procedure

From each of the acquired pieces of SVs, a biopsy sample 5 mm in length was taken and fixated by 4% buffered water formaldehyde solution. These samples were prepared using routine methods of preparation for the illumination microscope analysis. The tissue has undergone dehydration, illumination, and paraffin infiltration and was sliced by microtome into 4 nm sample sizes. These were subject to the methods of histochemical and immunohistochemical marking of specific tissue structures and finally brought to form permanent illumination microscope preparations. For immunohistochemical staining, anti-CD34 monoclonal antibody (mAb) (Dako; cat. No. M716501; dilution 1:50) was used and brought to the state of permanent illumination microscopy preparations in which the specific tissue bond is proven by secondary antibodies and EnVision-Flex visualizing system with a visible chromogen, later counterstained with Mayer hematoxylin. Microscopic preparations have been analyzed using illumination microscope digital camera Olympus BX50 (Olympus, Japan, Tokyo). Microscopic images of SV walls taken from the histochemical and immunohistochemical preparations were captured under constant magnification and stored digitally to be morphometrically processed. Morphometric analysis was achieved using the ImageJ 1.53 version (Wayne Rasband National Institute of Health, USA) program by quantifying the monoclonal antibodies binding rate to SV endothelium CD34 receptors. The degree of preservation of SVG endothelial integrity was determined based on the presence of CD34 positivity on the sections of the examined tissues.

SPSS statistical package (IBM, Armonk, New York, US), version 2.0, was used for statistical data analysis. The standard statistical method for quantitative and qualitative result assessment was used as a basic descriptive statistical parameter. Statistical significance was determined for $p < 0.05$. The statistic hypothesis was tested for a significance risk level value of $\alpha = 0.05$. The minimal sample size required to detect the effect size of 0.05 in the analysis of variance for the four groups and statistical power of 0.9 is 40 respondents.

Results

Table 2 and Figure 1 show the degree of preservation of SVG endothelial integrity, measured based on the CD34 mAb binding rate to the present endothelial cells in the examined tissue in all four groups concerning the type of solution used. Significant statistical difference in the presence of CD34 positivity (binding rate) expressed in percentages (CD34 % value) between the different used solutions groups was: $\chi^2 = 10.71$, df = 3, $p = 0.013 < 0.05$.

When the Shapiro-Wilk test showed deviation in CD34 % value in the whole sample and all tested solution groups, the Kruskal Wallis test was utilized to confirm the statistical significance of CD34 % value between the different solution groups ($\chi^2 = 10.71$, df = 3, $p = 0.013 < 0.05$). The following multiple group comparison, with the use of Bonferroni's correction, has confirmed the statistically significant difference in CD34 % value between the groups with FFP and Bretschneider's solution ($p = 0.008 < 0.01$), meaning that the difference in the CD34 % value is statistically significant in favor of the former.

The presence of T2DM did not make a significant statistical difference between the groups, and in the whole sample, 23 (57.70%) patients were burdened with T2DM (Figure 2).

Table 2

Degree of preservation of SVG endothelial integrity depending on the type of storage solutions

Solutions	CD34 (%)	
	presence	absence
Heparinized saline	73.5	26.5
Heparinized whole autologous blood	82.69	17.31
Fresh frozen plasma	92.9	7.1
Bretschneider's	26.77	73.23

SVG – saphenous vein graft. The number of patients in each tested group of solutions was 10.

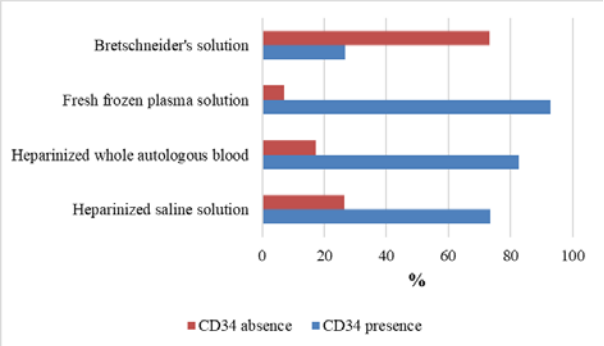

Fig. 1 – Statistical significance of CD34 % value between the different used solutions groups ($\chi^2 = 10.71$, df = 3, $p = 0.013 < 0.05$).

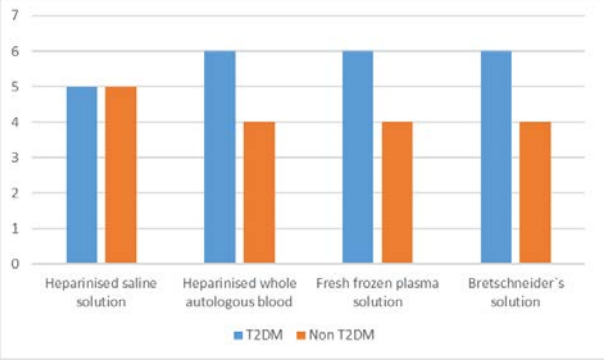

Fig. 2 – The distribution of type 2 diabetes mellitus (T2DM) with respect to the solution type and the entire sample. Data are presented in absolute numbers.

CD34 % value is slightly higher in the group of patients without T2DM, but based on data from the Mann-Whitney *U* test, no statistical significance was obtained (p = 0.7372) (Table 3).

In patients with T2DM, glycated hemoglobin (HbA1c) was recorded on a daily basis. Throughout the whole sample of patients, the value of Spearman's coefficient of correlation showed no significant correlation between the CD34 % value and the HbA1c parameter (ρ = -0.1901, p = 0.3850).

Histological examination

Several samples showed morphological presence of intimal tunic hypertrophy, which is, in some samples, of uniform development throughout the entire luminal circumference, and in others, present as a number of intimal cushions. Immunohistochemical staining with CD34 monoclonal antibodies and Mayer's hematoxylin counterstaining ensured clear visualization and intimal tunic differentiation between the cells with maintained luminal membrane integrity and the ones that showed endothelial layer denudation, as shown in Table 2. Figures 3 and 4 show (in magnification setting ×100) the difference in endothelial layer cell integrity and marked difference between the groups. In both Figures, the dark red-brown lining shows the presence of the endothelial cells, which have been marked using the ImageJ 1.53 version (Wayne Rasband National Institute of Health, USA) program by quantifying the mAbs binding rate to SV endothelium CD34 receptors, enabling morphometric analysis and visualization of endothelial layer integrity.

Table 3

Degree of preservation of SVG endothelial integrity depending on the presence of T2DM

Patients	CD34 (%)	
	presence	absence
T2DM	84.35	15.65
Non-T2DM	78.59	21.41

SVG – saphenous vein graft; T2DM – type 2 diabetes mellitus.

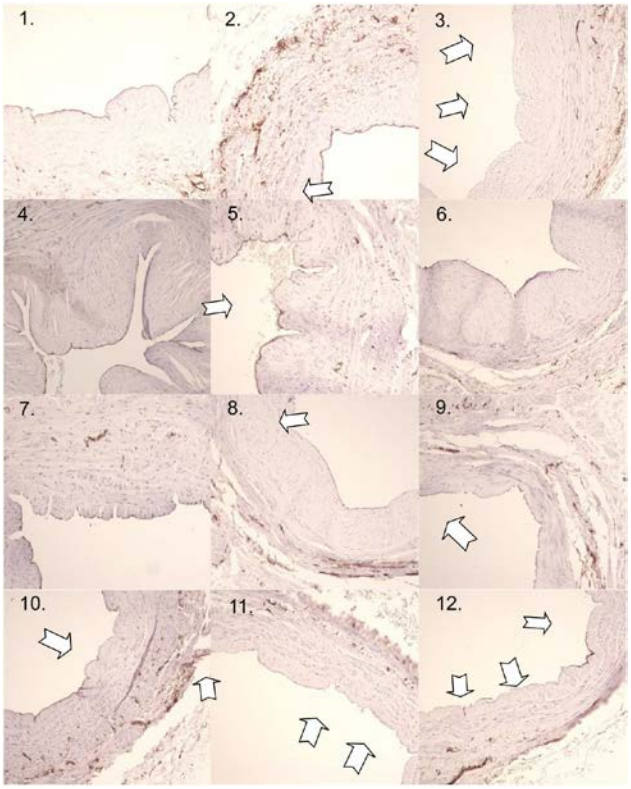

Fig. 3 – Immunohistochemical staining with anti-CD34 on saphenous vein graft (SVG). Counterstaining was performed using Mayer's hematoxylin. Arrows pointing at areas with endothelial layer denudation. Heparinized saline solution (1–3), heparinized whole autologous blood (4–6), fresh frozen plasma solution (7–9), and Bretschneider's solution (10–12) were used in SVG. Patients burdened with type 2 diabetes mellitus (photos 1, 2, 4, 5, 7, 8, 10, 11).

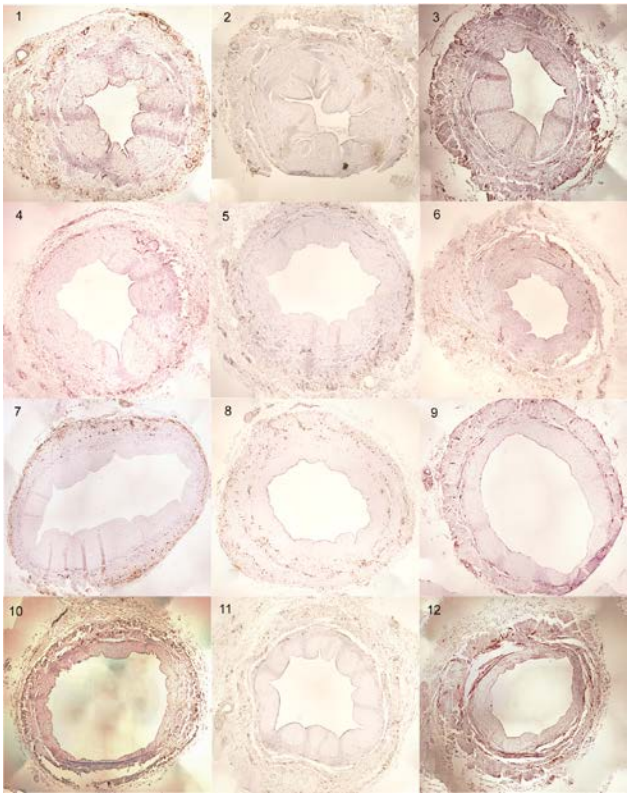

Fig. 4 – Immunohistochemical staining with anti-CD34 on whole cross sections of saphenous vein graft (SVG). Counterstaining was performed using Mayer's hematoxylin. Heparinized saline solution (1–3), fresh frozen plasma solution (4–6), heparinized whole autologous blood (7–9), and Bretschneider's solution (10–12) were used in SVG. Patients burdened with type 2 diabetes mellitus (photos 1, 2, 4, 5, 7, 8, 10, 11).

Discussion

The major findings of the present study are the following: 1) the FFP solution has shown to be superior to Bretschneider's solution when comparing SVG endothelial layer preservation properties ($p < 0.05$); 2) when comparing the two groups of patients, the presence of T2DM did not make a significant statistical difference in endothelial integrity preservation between the groups.

The difference between heparinized saline and heparinized whole autologous blood was not notable in this study in both groups of patients. The effect of the heparin itself remains to be examined further. It is known to distract some of the protective enzymes, relieving the oxidative stress and, therefore, making the endothelium more susceptible to different toxic influences [11, 12]. The first ever conducted study that relates storage solutions and SVG damage was conducted by Gundry et al. [13]. This group compared the effect of heparinized saline and autologous blood in temperatures 4–28 ˚C and dilation pressure from 100–300 mmHg. The use of heparinized saline solution created intramural edema independently from the distension pressure and, in combination with high distension pressure, led to massive endothelial damage. The authors concluded the utilization of heparinized whole blood in SVG storage and manipulation with dilation pressure under 100 mmHg to be an acceptable manner of treatment towards the SVG. Heparinized saline is the most economical solution and also has a reasonably positive effect on endothelial integrity preservation.

Studies have shown that heparinized whole blood may be more effective in preserving graft function compared to saline [14, 15]. Similar results were reported by Lawrie et al. [16] by analyzing SVGs harvested in conventional open technique. SVGs were also stored at room temperature but with higher dilation pressure (200–400 mmHg). Zerkowski et al. [17] compared heparinized autologous whole blood with human albumin, Bretschneider's solution, and heparinized saline and found the best results using autologous whole blood. Wilbring et al. [15] studied SV segments stored in heparinized saline and heparinized autologous whole blood submerged for 30 min at room temperature using Mulvany myograph, concluding heparinized saline is inferior to heparinized autologous whole blood, which does not correlate with the results from this study. These results are not confirmed by the morphometric measurements used in this study in both diabetic and non-diabetic patients; a contrast in results was found in favor of heparinized whole blood but not with statistical significance ($p > 0.05$). Similar to our results,

Chester et al. [18] have compared heparinized saline, heparinized whole autologous blood, and several commercial cardioplegic and storage solutions regarding endothelial reactivity and concluded there was no marked difference between heparinized saline and heparinized autologous whole blood.

In this study, the use of FFP was associated with a greater degree of endothelial preservation compared to the other solutions in both groups of patients using the conventional open-harvesting technique. Considering the FFP constituents match the soluble component of the endothelial glycocalyx, the fact can be utilized to achieve a potential beneficial gain in order to maintain the SVG endothelial cell integrity [19]. In 2010, Weiss et al. [20] continued research, confirming initial results from 2009 [9] and comparing them with ones gained from research with plasma derivative solutions. In both studies, the saline solution was found to be inferior.

Bretschneider's solution is a specialized mixture consisting of different components such as potassium, magnesium, and histidine. It is frequently utilized for the extended preservation of SVG and has the ability to maintain viability for up to 48 hrs [21]. The solution contains nutrients that help sustain the metabolic activity of SVGs, thereby enhancing graft patency. However, the use of Bretschneider's solution may lead to metabolic alkalosis and reduced myocardial contractility. In this study, Bretschneider's solution has been shown to be markedly inferior to the other solutions in the study, comparing the morphometric results obtained. Similar results were reported by Weiss et al. [9] in a study from 2009, showing that with the utilization of an FFP solution, the SVG tissue failed to maintain its integrity. By quantifying the CD34 monoclonal antibodies' binding rate to SV endothelium, the degree of endothelial layer integrity was determined. The clinical consequences of endothelial preservation vs. denudation cannot be foreseen precisely. The non-eventful adaptation of the SVG is not fully described on account of healthy grafts not being excised for ethical reasons. Analyzed samples are usually taken from non-functional grafts [22]. The endothelium's role is, among others, a vasoprotective one, and its dysfunction is recognized as a starting point for atherosclerosis [23]. Denudation is a factor in the development of atherosclerosis and mural thrombosis in animal models, and it also plays a role in plaque development. CABG clinical outcome is more important than graft patency in isolation. Denudation of the endothelium layer is unlikely to cause problems in the short term. The endothelium layer's capacity for regeneration may not, however, be sufficient in the long run [24]. Temperature alone could be a major factor in ischemia-reperfusion injury caused during harvesting. This study's findings are consistent with those of Unal et al. [25] from 2009 in that there was no significant statistical difference between storage solution temperature variations and CD34 binding rates. Although the average amount of time samples spent submerged in the storage solution ranged from 32.1 to 67 min, we did not find any statistically significant differences in the amount of time spent submerged. On the other hand, low storage solution temperature was identified by Lawrie et al. [16] as a risk factor for endothelial cell death.

Study limitations

One unit contains 250 mL of plasma and 500 mg of fibrinogen in a citrate anticoagulant, with an estimated albumin content of 26 g/L and 57 g/L of protein. The variation in FFP composition is present, and the effect of these variations is not known. The effect of FFP shelf life is also unknown. The influence of the harvesting technique is great, if not crucial, and cannot be compensated by even the "perfect" storage solution. Only two cross sections of each SV sample were processed.

Conclusion

There was a noticeable contrast in preserving SVG endothelial integrity between the two patient groups, diabetic (T2DM) and non-diabetic patients, although it was not statistically significant. The storage solution with the most beneficial effect on SVG endothelial integrity preservation was the FFP solution when harvested *via* the conventional open method in CABG.

Acknowledgement

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A cadaveric study of anatomical variations of the radial nerve and their clinical significance

Kadaverska studija anatomskih varijacija žbičnog živca i njihov klinički značaj

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Abstract

Background/Aim. The radial nerve (RN) is the largest terminal branch of the posterior cord of the brachial plexus. Upon leaving the axilla, the RN moves to the posterior compartment of the arm, where it makes close contact with the humerus. By penetrating the lateral intermuscular septum, RN enters the anterior compartment and, in the very proximity of the lateral epicondyle, divides into two terminal branches. The anatomy of this nerve is characterized by remarkable variability, the knowledge of which is of utmost importance in the fields of orthopedics and traumatology. The aim of the study was to examine the anatomy of the RN, including two parameters: the location and the way the RN divides into terminal branches, with a particular emphasis on the clinical implications of this data. **Methods.** The research was conducted on 27 cadavers, 15 female and 12 male, with a total of 54 upper extremities. After careful dissection, variations in the location and way of termination of the RN were observed on both the right and left hands. Collected data were then analyzed using Microsoft Office Excel. A classification where the division of the RN into terminal branches above the biepicondylar line (BEL) is defined as type A, while the division of RN below BEL is defined as type B was used. **Results.** According to the proposed classification, we observed a higher incidence of type A (66.7%) compared to type B (33.3%) in the total sample of 54 extremities. In addition, a higher prevalence of type A was observed in the female population, while a higher prevalence of type B was observed in the male population. There were differences in the distribution of types A and B between the left and right arms, but there were no variations in the way of termination of the RN. **Conclusion.** The present study showed an important complexity in the domain of RN anatomy with significant clinical implications. With that in mind, it is crucial for every patient that the limits of safe zones be defined while performing surgical procedures in the elbow to protect the RN and its branches from iatrogenic injuries.

Key words:
anatomy, regional; anatomic variation; cadaver; radial nerve; sex characteristics.

Apstrakt

Uvod/Cilj. Žbični živac (*nervus radialis* – NR) je najveća završna grana zadnjeg snopa ramenog živčanog spleta. Nakon što napusti pazušnu jamu, taj živac prelazi u zadnji region nadlaktice, gde stupa u bliski odnos sa ramenom kosti. Prolaskom kroz spoljašnju međumišićnu pregradu, NR ulazi u prednju ložu nadlaktice, da bi se na kraju, u predelu spoljašnjeg čvora ramene kosti, podelio na svoje dve završne grane. Anatomiju ovog živca odlikuje izuzetna varijabilnost, čije je poznavanje od velikog značaja u oblasti ortopedske i traumatske hirurgije. Cilj rada bio je da se ispita anatomija NR uključujući dva parametra: mesto i način podele NR na završne grane, sa posebnim naglaskom na kliničkom značaju ovih podataka. **Metode.** Istraživanje je sprovedeno na 27 kadavera, od kojih je 15 bilo ženskog a 12 muškog pola, sa ukupno 54 gornja ekstremiteta. Nakon pažljive disekcije, varijacije u mestu i načinu podele NR ispitane su i na levoj i na desnoj ruci. Analiza dobijenih podataka izvršena je pomoću kompjuterskog programa *Microsoft Office Excel*. Korišćena je klasifikacija po kojoj je podela NR na terminalne grane iznad biepikondilarne linije (BEL) definisana kao tip A, dok je podela NR ispod BEL definisana kao tip B. **Rezultati.** Prema predloženoj klasifikaciji, uočili smo veću zastupljenost tipa A (66,7%) u poređenju sa tipom B (33,3%) u ukupnom uzorku od 54 ekstremiteta. Osim toga, uočena je veća zastupljenost tipa A kod osoba ženskog pola, dok je kod muškaraca bio zastupljeniji tip B. Rezultati su pokazali i da postoji razlika u procentualnom

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udelu tipa A i tipa B između levih i desnih ekstremiteta ali varijacije u načinu završetka NR nisu bile utvrđene. **Zaključak.** Studija je pokazala značajnu složenost anatomije NR, sa važnim kliničkim posledicama. Imajući to u vidu, za svakog bolesnika neophodno je definisati granice „sigurne zone" prilikom hirurških operacija u regionu lakta, čime će biti obezbeđena zaštita NR i njegovih završnih grana od jatrogene povrede.

Ključne reči:
anatomija, regionalna; anatomija,varijacije; kadaver; n. radialis; pol, karakteristike.

Introduction

The radial nerve (RN) is the largest branch of the posterior bundle of the brachial plexus [1]. This mixed nerve provides motor innervation of the extensors of the entire upper extremity and sensory innervation of the back of the upper arm, forearm, and outer half of the back of the hand [2]. RN starts from the axillary fossa, passes to the back of the upper arm, and enters the radial groove on the back of the humerus, where it is followed by a deep brachial artery [3]. After breaking through the external intermuscular septum, the RN passes from the back to the front of the upper arm. It descends vertically to the external surface of the lateral epicondyle of the humerus, near which it ends by dividing into two branches [4]. The brachioradialis muscle covers the superficial, sensitive branch in the proximal part of the humerus. In contrast, the distal part is located under its tendon, passes to the back of the forearm, and becomes subcutaneous [5]. The deep, motor branch passes through the osteomuscular channel formed by the supinator muscle and the surrounding bony elements to finally end in the distal half of the forearm, having previously provided branches for the muscles of the back of the forearm [6].

The clinical importance of knowing the anatomy of an RN is invaluable. As previously mentioned, in the area of the upper arm, RN enters into a close relationship with the humerus, so it is not surprising that as many as 20% of all fractures of this bone are accompanied by injuries to the RN, which, fortunately, are usually of a mild degree, pass spontaneously and do not require surgical treatment [7, 8]. In addition to the mentioned primary lesions, there are also frequent secondary iatrogenic lesions of the RN that occur during surgical interventions to repair complicated humerus fractures [8]. In the region of the cubital fossa, the RN divides into two final branches. At this point, it is highly susceptible to injury during the anterior-external approach to the elbow joint [9]. In addition to orthopedic surgery and traumatology, knowledge of the anatomy of the RN has found practical applications in other fields of medicine. Thus, for doctors specializing in neurology, it is imperative to precisely define the pattern of separation of the motor and sensory branches of RN so that, based on clinical examination, they can determine the level of the lesion in chronic compressive neuropathies, such as radial tunnel syndrome [10]. As the clinical anatomy of the RN is directed towards a wide range of medical specialties, it should not be surprising that a large number of cadaveric and radiographic studies of the RN have been conducted. Most researchers' focus of interest was the RN position in the area of the upper arm (*regio brachii*). These studies aimed to define and precisely determine the boundaries of the 'safe zone' during surgical interventions in the region of the distal part of the humerus to minimize the possibility of iatrogenic injury to this nerve [11]. For this purpose, various anatomical and topographic landmarks were used, which served as reference points in relation to which distance of RN was measured, expressed in absolute or relative numbers [12]. It is important to note that, despite efforts to clearly define the path and relationships of RN, the segment of anatomy related to the place of the division of RN into its final branches remains unexplored. To the best of our knowledge, this study is among the first to examine the anatomical variations of the final part of RN. This study aims to examine the anatomy of the RN in the region of the cubital fossa, which will include two parameters: 1) the determination of the place and 2) the manner of termination of the RN, with a particular emphasis on the clinical significance of the results.

Methods

Our research was conducted on cadaveric material of the Institute of Anatomy "Niko Miljanić", Faculty of Medicine, University of Belgrade, with the approval of the Ethics Committee for the use of human and laboratory animal material, Faculty of Medicine, University of Belgrade, Serbia (No. 325-07-01245/2014-05/2). The study included a total of 27 cadavers, of which 12 were male and 15 female, aged from 64 to 79 years. Initially, a visual check of each extremity was performed to exclude samples with deformities and traces of trauma or surgery. After fixation in formalin solution, the extremities were carefully dissected, following standard procedures from dissection manuals. For our study, two groups of samples were formed, which included 54 upper extremities, of which 24 were in the male group and 30 in the female group. The first aim of the examination was to define the place where the RN terminates and divides into its final branches, the superficial and deep branches. Based on previous research [1–6], it was hypothesized that RN can end in two ways, which we defined as type A and type B (Figure 1). The division of the RN into terminal branches above the biepicondylar line (BEL) (Hueter's line – an imaginary line passing through the most prominent points of the lateral and medial epicondyle of the humerus, named after the author) when the forearm is in a position of extension and supination is defined as type A, while the division of the RN below the BEL is defined as type B. All parameters were measured bilaterally. The measuring instruments used in the research were a ruler and an electronic digital caliper (measuring range 0–500 mm, resolution 0.01 mm). The study's second aim was the manner of termination of the RN, defined as the number of terminal branches into which this nerve is

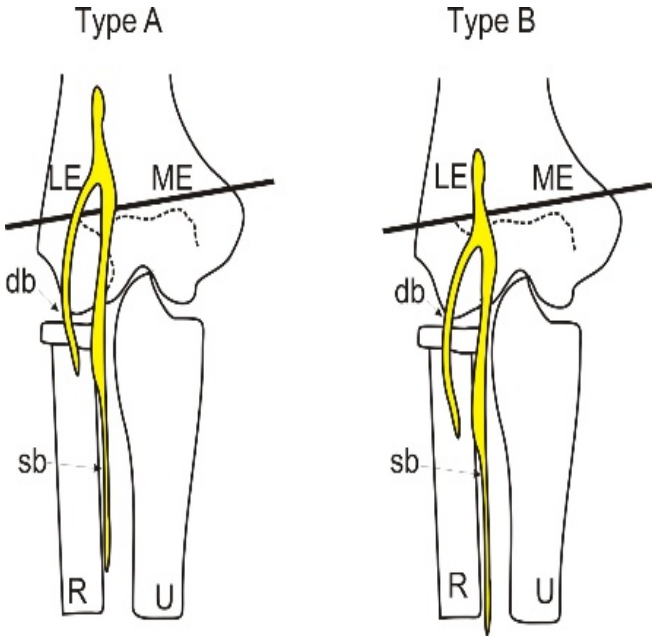

Fig. 1 – Schematic view of the division of the radial nerve (RN) above (type A) and below (type B) the Hueter's biepicondylar line (bolded black line). db – deep branch of RN; sb – superficial branch of RN; LE – lateral epicondyle; ME – medial epicondyle; R – *radius*; U – *ulna*.

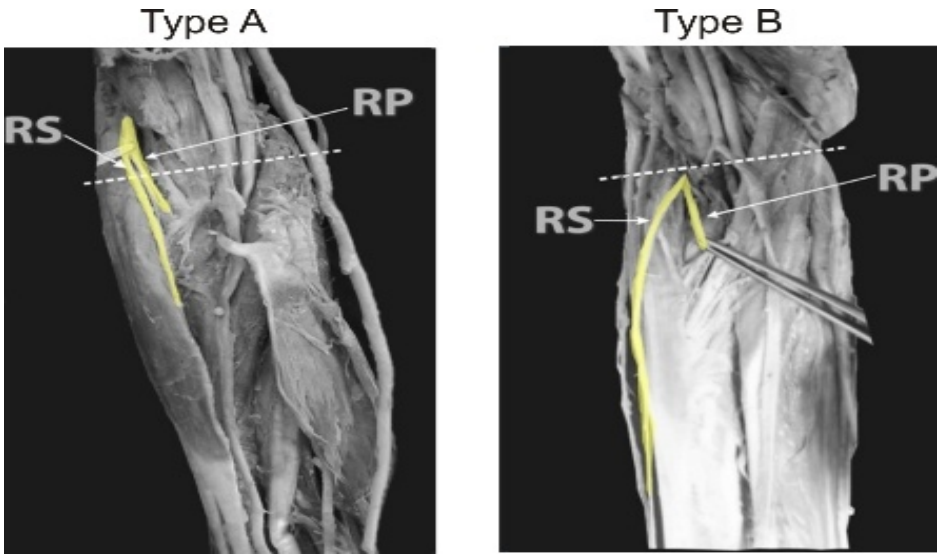

Fig. 2 – Example of dissected cadaveric material which shows the division of the radial nerve (RN) above Hueter's line (type A) and division of the RN below Hueter's line (type B) (dashed line). RS – *ramus superficialis* (superficial branch of RN); RP – *ramus profundus* (deep branch of RN).

divided. The research methodology is shown schematically (Figure 1), while the results of examined parameters are shown in Figure 2.

Results

The total number of examined cadavers was 27, of which 12 were male and 15 were female, and the investigation was conducted on both arms of each cadaver (total of 54 arms). The prevalence of type A of RN termination (point of division) was observed in 36/54 extremities, representing 66.7% of the total examined sample. In comparison, division point according to type B was found in 18/54 extremities, representing 33.3% of the total sample. The results of our research are presented in Table 1. The goal of further processing of the data was to examine the distribution by gender for both types of division points of the RN and whether there are any differences in the frequency of type A or type B between the left and right extremities. Thus, in the group of male cadavers, type A was observed in 14 (58.3%) limbs and

type B in 10 (41.7%) limbs. In this group, no differences in frequency between the left and right extremities were found, meaning type A and type B occurred equally often on both extremities. In the group of female cadavers, type A was found in 22 (73.3%) limbs and type B in only 8 (26.7%) limbs. Here, variations in frequency between the left and right extremities were observed, with type A occurring more often on the left side (2:1 ratio) and type B occurring more often on the right side (3:1 ratio). On all of the preparations that made up the sample material, the division of the RN into superficial and deep ending branches was established as the only way of termination of the RN.

Table 1

Prevalence of type of the radial nerve (RN) division obtained from extremities of cadavers

Parameter	Type A	Type B
Male (n = 24)	58.3	41.7
Female (n = 30)	73.3	26.7
Total (n = 54)	66.7	33.3

Results are shown as a percentage of prevalence. Type A – division point of the RN above the Hueter's line; type B – division point of the RN below the Hueter's line; n – number of extremities.

Discussion

RN is one of the largest and longest nerves of the upper limb, with an extensive motor and sensory innervation field. This nerve, among other things, is characterized by extraordinary diversity regarding the place and manner of division into its two terminal branches, superficial and deep.

As it descends, the RN enters into significant anatomical relations with the surrounding structures, a good knowledge of which is of immense importance in everyday clinical practice. By reviewing the available literature, we found many studies and articles where the manner of its origin, path, and relations with adjacent bony and blood vascular elements (humerus and deep brachial artery) are described in detail [11–14]. Studies also described the place and the level of separation of the motor branches [6, 15] and the anatomical varieties of its sensitive end branch [5, 16–18]. However, the precise determination of the location of the RN's final division remained in the shadow of the aforementioned research. Defining where the RN ends can also be found in standard anatomy textbooks, in which it is briefly described that the RN ends in the immediate vicinity or in front of the lateral epicondyle of the humerus [3].

Considering all the facts mentioned above, the main task of our study was to shed new light on this important aspect of the anatomy of the RN. The first goal of our study was to examine the exact place of division, i.e., termination of RN. We presumed, which was confirmed during the examination itself, that the place of the division of the RN could be found above or below the BEL. Based on the obtained results, we proposed two types of termination of the RN: type A (above BEL, i.e., high division) and type B (below BEL, i.e., low division). One of the first studies that dealt with the problem of variations in the termination of the RN was conducted by Fuss and Wurzl [13] in 1991. They showed that the site of division of RN can be found anywhere within a 5.5 cm segment extending from 2.5 cm above BEL to 3 cm below BEL [13]. Similar results were obtained by Artico et al. [19], who measured the average distance between the division site of RN and the lateral epicondyle, which was 2.9 cm. The mentioned studies [13, 19] did not analyze the frequency of high or low location of division of RN in the total population, nor its structure according to gender. In the present study, we reached three important conclusions regarding the frequency of different types of division sites of the RN. First, the entire sample material showed a higher prevalence of type A (66.7%) compared to type B division location of RN (33.3%). By further studying the data by gender, it was observed that in both genders, type A of RN is found more often in regard to the total sample of 54 limbs, but in females, type A occurs more often than in males. The obtained results only partially agree with the study conducted by Sapage et al. [1], which established that gender is not a factor that determines where the RN will divide into its final branches. The last level of analysis involved examining differences in the frequency of type A and B between the left and right extremities in both genders. In the group of male cadavers, the mentioned differences were not observed, i.e., high and low types of division of RN occurred equally often on both sides. On the other hand, in the group of female cadavers, the following rule was established: type A occurred more often on the left side, while type B of the division occurred more often on the right side. Next, when we asked what is the basis of the anatomical variations of the place of termination of the RN (point of division of this nerve into two final branches), there was still no adequate answer. However, two assumptions may be helpful on the way to finding the answer. According to the first assumption, the key factor that determines the type of division we encounter in adulthood (high or low) is disproportion, i.e., the disproportion in the growth rate of the long bones of the upper limb on the one hand and the rate of elongation of the nerve elements during the development phase on the other [20]. The second assumption, probably even more interesting than the first one, is that the dominance of the hand, i.e., the characteristic of whether one is right-handed or left-handed, can also condition the very place of the division of the vertebral nerve, as is the case with the palmar arterial arches [21]. In the future, it is necessary to carry out adequate tests to prove or disprove the mentioned hypotheses. Another aim of our study was to determine the manner of termination of the RN. This parameter is defined as the number or type of terminal branches of the RN. On a total of 54 observed limbs, the division of the RN into two final branches – the superficial (sensory) and the deep (motor) branch – was established as the only way of termination, which is in accordance with the description that can be found in anatomy textbooks [2, 3]. Yet, there is extensive data in the literature about numerous variations regarding the way of termination and the number of final branches of the RN. Thus, cases with three terminal branches (superficial, deep, and the branch in-

tended for the long external extensor of the hand) have been described [22], with only one terminal branch (superficial, the sensory branch is omitted) [13], with the doubling of the surface branch of the RN [17] and with the separation of the branches intended for the upper arm muscle [23], which is predominantly innervated by the median nerve. The mentioned variations, which are extremely rare, were not observed in the present study, probably due to the small sample size [1] and the existence of ethnic differences between the study populations [2, 24–26].

Finally, it is necessary to point out that knowledge of anatomical variations when it comes to the point of the division of RN into its final branches is crucial, especially in the field of orthopedic surgery [27]. The main aim and purpose of a careful and dedicated study of the anatomy of the RN is to avoid injury to this nerve during surgical interventions in the elbow region (*regio cubiti*). Nowadays, this is considered essential, bearing in mind the constant strive to improve the safety of all medical procedures, including surgical ones. Namely, due to the high sensitivity of nerve structures and the low capacity for regeneration (especially if there is neurotmesis, i.e., complete transection of the nerve), any injury to the RN can leave far-reaching negative consequences for the patient's health [28]. That is especially true for those cases where there is a type B division of RN (i.e., low division) when, in case of a nerve lesion, there would be an outage of both the sensory and motor components, with the loss of extension of the hand and fingers ("hanging hand", a cardinal sign of injury of the RN) as well as the loss of sensitivity on the back of the forearm and the outer half of the back of the hand, with the appearance of painful paresthesias. The probability that an iatrogenic lesion will occur depends on several factors and, above all, on the nature of the disease itself, which needs to be solved surgically because the nature of the disease affects the applied surgical approach [29, 30]. Thus, the lowest risk will exist with the posterior approach (through which complicated fractures of the distal part of the humerus bone are solved). In contrast, a significantly greater risk will exist with the anterior and anterior–lateral approaches, which serve for the repair of complicated fractures in the area of the elbow joint (through open reduction and internal fixation), for arthrocentesis (to treat joint infection), total arthroplasty and treatment of compressive neuropathies of the RN [9]. In the end, it is crucial for each patient to preoperatively define the limit of the 'safe zone' in the region of the elbow joint in order to avoid injuries to key anatomical structures in this region, including the RN.

Conclusion

In order to acquire detailed information on all possible variations of the final part of RN, in the present study, a new system was proposed that includes type A and type B divisions of the RN. The results have shown a higher prevalence of type A in the total sample, but certain differences in frequency depending on gender and laterality were also recorded. The obtained results, although significant as such in the field of orthopedic surgery, indicate the necessity of further research in this area to more precisely define the safe limits during surgical interventions in the elbow joint area.

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The impact of dental varnishes on the immediate surface microhardness and roughness of restorative dental materials: an *in vitro* study

Uticaj dentalnih lakova na mikrotvrdoću i hrapavost površine restaurativnih materijala: *in vitro* studija

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Abstract

Background/Aim. Dental caries is a multifactorial disease that ultimately results in the demineralization of dental tissues and is recognized by the World Health Organization as the most prevalent disease among the global population. Dental varnishes are effective in preventing caries in children. The aim of this study was to investigate the way in which dental varnishes, one of which contained fluoride and the other casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), affect the microhardness and roughness of the three most commonly used restorative materials in pediatric dental practice [resin-modified (RM) glass ionomer cement (GIC), high-viscosity (HV) GIC, and micro-hybrid composite (MHCOMP)]. **Methods.** The study included 60 discs and 60 bars, of which 20 discs and 20 bars each were made from one of the three commonly used dental restorative materials. After preparation, incubation, and subsequent basic measurement, the samples were divided into two subgroups (each subgroup containing 30 discs and 30 bars), and each of them was treated with fluoride or CPP-ACP varnish according to a precisely established protocol. After treatment with varnishes, all samples were retested for microhardness and roughness. **Results.** The application of fluoride or CPP-ACP varnish increased the microhardness of the MHCOMP and RM GIC but reduced the microhardness of HV GIC. The roughness was more pronounced with the composite material, as well as with the RM GIC. **Conclusion.** Prophylactic varnishes containing fluoride and calcium have the potential to alter the microhardness and roughness of dental restorations; that is why carefully selecting the appropriate varnish is important.

Key words:
child; dental cements; fluorides; preventive dentistry.

Apstrakt

Uvod/Cilj. Dentalni karies je multifaktorska bolest koja za krajnji rezultat ima demineralizaciju zubnog tkiva, a Svetska zdravstvena organizacija ga je označila kao najrasprostranjeniju bolest u svetskoj populaciji. Dentalni lakovi su se pokazali efikasnim u prevenciji karijesa kod dece. Cilj rada bio je da se istraži način na koji zubni lakovi, od kojih je jedan sadržao fluor a drugi kazein fosfopeptid-amorfni kalcijum fosfat (KFP-AKF), utiču na mikrotvrdoću i hrapavost tri najčešće korišćena restaurativna materijala u pedijatrijskoj stomatološkoj praksi [smolom modifikovani stakleno-jonomerni cement (SJC), visokoviskozni SJC i mikrohibridni kompozit (MHKOMP)]. **Metode.** U studiju je bilo uključeno 60 diskova i 60 pločica, od kojih je po 20 diskova i 20 pločica bilo napravljeno od jednog od ukupno tri restaurativna materijala. Uzorci su nakon pripreme, inkubacije i sledstvenog bazičnog merenja, podeljeni u dve podgrupe (sa po 30 diskova i 30 pločica), od kojih je svaka tretirana fluoridnim ili KFP-AKF lakom, prema tačno utvrđenom protokolu. Nakon tretiranja lakovima, na svim uzorcima je ponovo testirana mikrotvrdoća i hrapavost. **Rezultati.** Primenom fluoridnog ili KFP-AKF laka povećala se mikrotvrdoća MHKOMP i smolom modifikovanog SJC, ali se smanjila mikrot

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vrdoća visokoviskoznog SJC. Hrapavost je bila izraženija kod kompozitnog materijala, kao i kod smolom modifikovanog SJC. **Zaključak**. Profilaktički lakovi koji sadrže fluor i kalcijum imaju potencijal da promene mikrotvrdoću i hrapavost zubnih nadoknada i zbog toga je veoma važno pažljivo izabrati odgovarajući lak.

Ključne reči:
deca; zub, cement; fluoridi; stomatologija, preventivna.

Introduction

Dental caries, affecting both deciduous and permanent teeth, is recognized as the most prevalent disease among the global population, as reported by the World Health Organization [1]. This multifactorial disease ultimately results in the demineralization of dental tissues. Glass ionomer cement (GIC) is commonly employed as a filling material in dentistry to address both early-stage and cavitated carious lesions in deciduous and permanent dentition [2]. Moreover, their fluoride content and subsequent release enable them to exhibit a cariostatic effect, aiding in the prevention and control of dental caries [3]. Additionally, composites are widely utilized as restorative materials for the treatment of both deciduous and permanent dentition rehabilitation. The wear of light-curing composite resin is influenced by multiple factors, including the material's physical properties, microstructure, resistance to abrasion, and the impact of masticatory forces [4]. Conversely, the wear of GIC represents one of the drawbacks associated with this material [5].

Prophylactic varnishes containing fluoride and calcium are widely employed for the prevention of initial carious lesions following timely diagnosis [6]. Several studies have demonstrated the effectiveness of topical fluoride application in reducing caries incidence [7]. A crucial role of fluoride varnishes lies in the formation of fluorapatite, a compound that exhibits resistance to acidic environments and demineralization. These varnishes can effectively adhere to dental surfaces, ensuring long-term and controlled fluoride release [8].

Amorphous calcium phosphate (ACP) serves as a precursor for both hydroxyapatite and casein phosphopeptide (CPP) [9]. It is noteworthy that ACP represents the first commercially available artificial hydroxyapatite product [10].

The literature suggests that the application of topical varnishes enriched with fluorides and CPP-ACP particles, followed by their incorporation into GIC, can influence the mechanical properties of the material. Specifically, it can affect the surface microhardness and roughness. Some studies have reported a decrease in microhardness, while others have observed an increase when using different restorative materials [11–13].

The primary aim of this study was to determine the microhardness of three restorative materials that we use in our daily practice, and the secondary aim was to compare the effect of varnishes containing fluoride, calcium, and phosphate on the microhardness and roughness of these dental materials.

Methods

The study adheres to the principles outlined in the Helsinki Declaration and has obtained approval from the Ethics Committee of the Faculty of Medicine, University of Banja Luka, Republic of Srpska, Bosnia and Herzegovina, reference Number 18/4.17/23.

For the measurement of microhardness, samples were prepared using pre-made molds (Figure 1).

The molds were used to create discs with a diameter of 15 mm and a thickness of 2 mm. The roughness measurement samples were created using a bar-shaped mold with dimensions of 30 mm × 2 mm × 2 mm (Figure 2).

A total of 120 samples were prepared, comprising 20 discs and 20 bars for each of the three dental materials used: resin-modified (RM) glass ionomer cement (GIC) (Fuji II LC®, GC Corporation Tokyo Japan), high-viscosity (HV) GIC (FUJI IX GP®, GC Corporation Tokyo Japan), and micro-hybrid composite (MHCOMP) Te-Econom Plus® (Ivoclar Vivadent, Schaan, Liechtenstein). To prevent the materials from sticking and ensure easy removal from the mold, the walls of the molds were coated with a layer of soft paraffin.

Fig. 1 – Sample molds.

Fig. 2 – Material samples, in the shape of discs and bars.

RM GIC and HV GIC samples were precisely prepared according to the manufacturer's instructions. After mixing, RM GIC and HV GIC were introduced into the molds with a spatula so that a glass plate was pressed from the bottom side of the mold, not allowing the material to leak, and after filling the mold, the glass plate was also pressed from the top by its weight. The HV GIC was cured at room temperature for 15 min, according to the manufacturer's instructions, and RM GIC was polymerized by the light-curing lamp (Woodpecker LED.B, 1,200 mW/cm^2, wavelength: 385 nm – 515 nm, Guilin Medical Instrument Co., Ltd.) in direct contact with the material surface. Similarly, the MHCOMP was introduced into the mold, and the mold was secured with glass plates to maintain proper positioning. The composite material was then polymerized by direct contact with a light-curing lamp for 20 sec. That ensured thorough and adequate polymerization of the composite [14]. All samples underwent polishing and fine instrument processing (3M Espe, Sof-lex XT, Pop-On Polishing Discs 12.7 mm) following the standard protocol for restorative fillings on teeth. This step ensured that the samples were appropriately finished and prepared for further analysis.

Following their fabrication, all the discs were placed in plastic containers containing 2 mL of distilled water [12]. The containers were then incubated at a temperature of 37 °C for two days. This step aimed to simulate the conditions found in the oral cavity, allowing for potential interactions and changes that may occur over time. On the other hand, the lack of thermocycling of restorative materials is also another caveat of the study.

After the incubation period, the samples were carefully dried and transported to the laboratory. The microhardness was determined using the Vickers method (Emco Test GmbH, Dura Scan 20 G5, Figure 3).

During the microhardness testing process, a diamond pyramid with a square base and a tip angle of 136° was used as an indenter, and a load of 100 g was applied for 15 sec. Measurements were taken at three different points on each sample within a 15 mm diameter area. The average value of these measurements was then considered as the baseline microhardness value for each sample. Roughness was measured by contact profilometry using a Mitutoyo SJ-310 (Mitutoyo Corp., Kawasaki, Japan) profilometer (Figure 4).

The measuring sensor that slides along the examined profile had a measuring probe with a diameter of 2 μm under the action of force F = 0.7 mN.

Each material was divided into two subgroups (10 samples each), of which the first subgroup was treated with fluoride varnish [Fluor (F) protector S, 7,700 ppm, 1.5%], and the second was treated with MI Varnish™ (5% sodium-fluoride, 22,600 ppm, CPP-ACP).

The same procedure for determining the microhardness and roughness of the samples after applying the varnish was repeated in the laboratory. The obtained values are presented in the form of statistical data.

Statistical processing and analysis were done in the SPSS version 24. The alpha value was established at $p < 0.05$.

Frequencies and percentages were used to describe important parameters, while the median (Me) with interquartile range (IQR) was used to describe numerical variables. Differences in two time intervals were tested by Wilcoxon Test.

Results

The microhardness of HV GIC material was statistically significantly different after MI Varnish™ application (Me = 44.55; IQR = 2.85) compared to baseline values (Me = 72.10; IQR = 5.03), $p < 0.001$, but also after F protector S application (Me = 36.15; IQR = 2.85), $p < 0.001$. A statistically significant difference compared to the baseline measurement was also recorded when it comes to the MHCOMP material after the MI Varnish™ application ($p < 0.001$) and after the F protector S application ($p < 0.001$). Differences were noted in the microhardness of the RM GIC material after the MI Varnish™ application ($p < 0.001$), as well as after the F protector S application ($p < 0.001$).

The microhardness of HV GIC, MHCOMP, and RM GIC was statistically significantly different in baseline measurement (Me = 24.75; IQR = 0.82), in comparison to

Fig. 3 – Vickers microhardness device.

Fig. 4 – Mitutoyo SJ-310 profilometer.

the measurement after MI Varnish™ (Me = 43.00; IQR = 3.35) or F protector S application (Me = 52.5; IQR = 1.93), $p < 0.001$. The results are shown in Figure 5.

The roughness of HV GIC material is statistically significantly different when comparing baseline measurement (Me = 0.26; IQR = 0.03) to the measurement performed after MI Varnish™ (Me = 1.13; IQR = 0.11) or F protector S application (Me = 0.91; IQR = 0.16), $p < 0.001$. A statistically significant difference was also noted concerning the MHCOMP material ($p < 0.001$). The roughness of this material was lowest in the baseline measurement (Me = 0.80; IQR = 0.10), statistically significantly higher after MI Varnish™ (Me = 1.23; IQR = 0.12) or F protector S application (Me = 1.22; IQR = 0.13), $p < 0.001$. Differences were noted in the roughness of the RM GIC material after MI Varnish™ (Me = 0.33; IQR = 0.08) and F protector S application (Me = 1.07; IQR = 0.16), $p < 0.001$.

The roughness of all materials was statistically significantly different in baseline measurement (Me = 0.33; IQR = 0.08) compared to measurement after MI Varnish™ application (Me = 1.24; IQR = 0.12), and after F protector S application (Me = 1.07; IQR = 0.16), $p < 0.001$. The results are shown in Figure 6.

Discussion

In this study, we observed that the addition of MI Varnish™ and fluoride varnish leads to differences in the microhardness and roughness of the restoration materials. Similar findings are found in previous studies, examining different

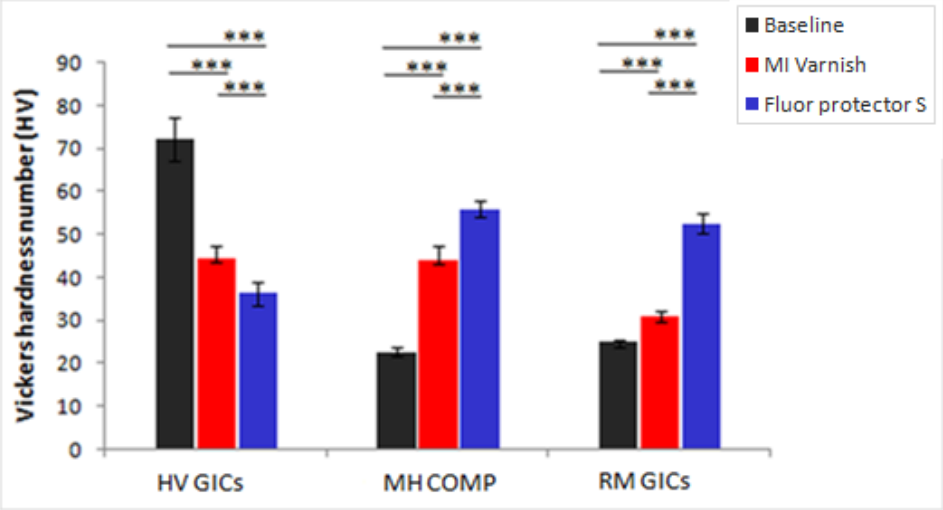

Fig. 5 – Microhardness of the high-viscosity (HV) glass ionomeric cement (GIC) Fuji IX GP®, micro-hybrid composite (MHCOMP) Te-Econom Plus® and resin-modified (RM) GIC Fuji II LC®. Baseline measurement compared to measurement after application of MI Varnish™ or Fluor (F) protector S (*$p < 0.001$).**
Results are shown as median and interquartile range. Wilcoxon test was applied.

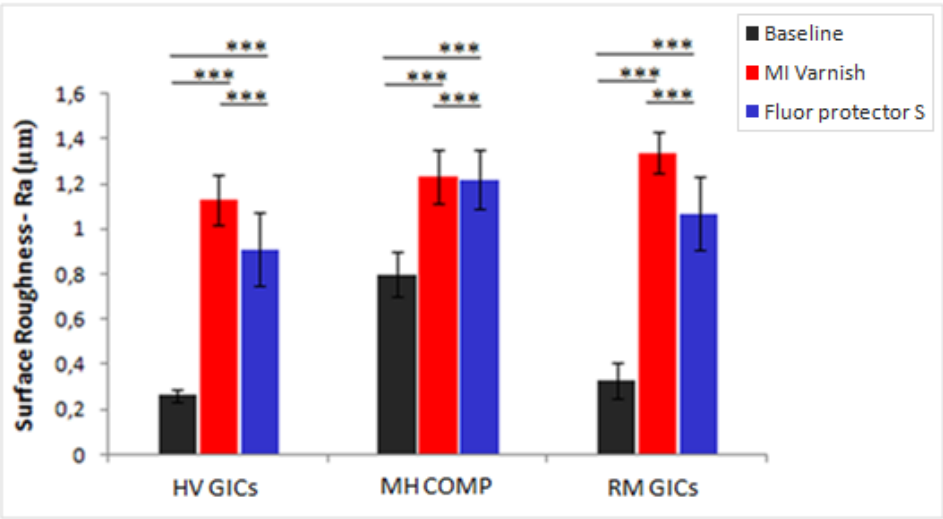

Fig. 6 – The roughness of the HV GIC Fuji IX GP®, MHCOMP Te-Econom Plus®, and RM GIC Fuji II LC®. Baseline measurement compared to measurement after application of MI Varnish™ or F protector S (*$p < 0.001$).**
Results are shown as median and interquartile range. Wilcoxon test was applied.
For abbreviations, see Figure 5.

dental varnishes [15]. HV GICs are materials composed of fluor aluminosilicate glass and polyacrylic acid. These types of cement have a higher powder-to-liquid ratio, specifically a higher proportion of polyacrylic acid to the powder. As a result, they exhibit greater resistance compared to standard glass ionomers [16]. Both HV and RM types of GIC have a disadvantage when it comes to their dissolution in an acid-base environment [17]. This means that these types of cement may experience degradation or erosion when exposed to acidic or alkaline conditions, which can affect their longevity and effectiveness as dental restorative materials. GIC restorations are biocompatible with dental structures and act as a fluoride ion reservoir, aiding in the remineralization process. The addition of CPP-ACP varnish over glass ionomer restorations enhances their effectiveness in preventing caries. Studies have demonstrated the efficacy of applying MI Varnish™, which contains 5% sodium fluoride or CPP-ACP, to damaged enamel surfaces. This application increases the release of calcium, fluoride, and phosphate ions onto the tooth surface, promoting remineralization [18]. Fluoride protector 0.7% is a highly soluble solution that facilitates the formation of fluoride deposits, aiding in the remineralization of the tooth surface by incorporating fluoride ions into the crystal lattice [19]. In this study, we observed that the addition of MI Varnish™ and fluoride varnish led to a decrease in the microhardness of HV GIC. Similar findings regarding the reduction in microhardness of HV GIC after the application of fluoride varnishes and the addition of CPP-ACP have been reported in previous literature [12]. Potential adverse effects of dental varnish application might be material dependent [20], as in our study. Conversely, some studies have indicated that fluoride, calcium, and phosphate ions can increase the microhardness of RM GIC [21]. F protector S varnish, containing 1.5% ammonium fluoride, ethanol, and water, has a greater impact on the microhardness of RM GIC compared to MI Varnish™. After the evaporation of the alcoholic component, the concentration of fluoride on the treated surface is approximately ten times higher [22]. The formation of a fluorapatite compound, which is more resistant to dissolution than hydroxyapatite, is one possible explanation for the increase in microhardness observed in the treated material. Te-Econom Plus® composite contains bisphenol A-glycidyl methacrylate (Bis-GMA), urethane dimethacrylate, and triethylene glycol dimethacrylate (TEGDMA), which makes it an aesthetically pleasing and mechanically robust restorative material. In this study, we observed a significant increase in microhardness with the application of F protector S varnish and a slight increase with MI Varnish™. Previous literature has reported a decrease in microhardness when using pH-modifying compounds [23]. However, our study utilized different topical varnishes, which may yield varying effects on the material [24]. Some studies have shown an increase in microhardness after the application of fluoride-based varnishes [25]. Additionally, it is worth considering that the surface polishing of the composite samples involved partial coverage with paraffin used for insulation, which could contribute to the observed increase in microhardness [26]. This is a potential caveat of the study.

After treatment, the roughness of all samples showed an increase [27]. The MI Varnish™, due to its density, required physical removal from the sample. Even after drying with a duster, the remaining residue had to be carefully dried with cotton balls to ensure accurate profilometer measurements, similar to previous studies [28]. While increased roughness is an undesirable characteristic that facilitates the adhesion of food and bacteria to the material surface, the benefits of fluoride and calcium outweigh this concern, especially for patients at risk of caries [29].

The surface microhardness of restorative dental material increases after fluoride varnish application. Although an increase in the surface roughness of restorative dental material is an undesirable effect of this varnish application, the formation of fluorapatite and remineralization of demineralized enamel outweigh this risk.

The limitation of this study is that the use of paraffin as an insulator should be quantified in the following research on the microhardness of the materials. Furthermore, the lack of use of thermocycling could be a caveat, and hence direct extrapolation of these results in a clinical setting is not possible.

Conclusion

This study provides evidence that varnishes such as F protector S and MI Varnish™ can affect the microhardness and roughness of restorative materials. Despite the limitations of the study, which is reflected in the use of paraffin as an insulator, these findings highlight the importance of considering material compatibility when selecting varnishes for different types of dental restorative materials.

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Use of lung ultrasound in the differential diagnosis of the causes of dyspnea

Primena ultrazvuka pluća u diferencijalnoj dijagnozi uzroka dispneje

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Abstract

Background/Aim. The field of lung ultrasonography (US) is a fast-developing one, and it provides the medical community with numerous new diagnostic opportunities. The aim of this study was to examine the etiology of dyspnea on admission and assess the stage of heart failure (HF) according to the US examination of the heart and lungs. **Methods**. The cross-sectional study included a total of 110 patients treated for the symptoms of dyspnea. The study included all patients treated for any heart or pulmonary condition, as well as patients who reported the first episode of dyspnea without any previous illness. The most important diagnostic sign in the US of the lungs in patients with HF was the appearance of B-lines or "comets" (ultrasound artifacts reminiscent of comet tails), which indicate the accumulation of fluid in the interstitium of the lungs. **Results**. The mean number of registered "comets" in the total number of patients was 14.2 ± 7.4 (minimum 2, maximum 30). The mean number of "comets" among patients with HF was 18.8 ± 5.9. The mean number of "comets" among patients without HF was 8.0 ± 3.7 ($p < 0.001$). Multivariate linear regression analyses showed the association between the number of "comets" and HF ($p < 0.001$). **Conclusion**. As the assessment of present "comets" in pulmonary US examination is safe and non-invasive, it can easily be integrated into the daily clinical practice because it has been shown that the number of pulmonary "comets" is significantly higher in patients with HF compared to those with dyspnea of other etiologies.

Key words:
diagnosis, differential; dyspnea; heart failure; pulmonary edema; ultrasonography.

Apstrakt

Uvod/Cilj. Polje primene ultrazvuka (UZ) kod pregleda pluća se brzo razvija i pruža medicinskoj zajednici mnogobrojne nove dijagnostičke mogućnosti. Cilj rada bio je da se na osnovu UZ pregleda srca i pluća ispita etiologija dispneje pri prijemu bolesnika i proceni stepen slabosti srca (SS). **Metode.** Studijom preseka obuhvaćeno je ukupno 110 bolesnika lečenih zbog dispneje. Obuhvaćeni su svi bolesnici lečeni zbog bilo koje srčane ili plućne bolesti, kao i bolesnici koji su prijavili prvu epizodu dispneje bez bilo kakvog prethodnog oboljenja. Najvažniji dijagnostički znak na nalazu UZ pluća kod bolesnika sa SS bila je pojava B linija ili „kometa" (artefakti koji podsećaju na repove kometa), koje ukazuju na nakupljanje tečnosti u intersticijumu pluća. **Rezultati.** Srednji broj registrovanih „kometa" kod ukupnog broja bolesnika bio je 14,2 ± 7,4 (minimum 2, maksimum 30). Srednji broj „kometa" kod bolesnika sa SS bio je 18,8 ± 5,9. Srednji broj „kometa" kod bolesnika bez SS bio je 8,0 ± 3,7 ($p < 0,001$). Multivarijantna linearna regresiona analiza pokazala je povezanost između broja „kometa" i postojanja SS ($p < 0,001$). **Zaključak.** S obzirom na to da je procena prisustva „kometa" tokom UZ pregleda pluća bezbedna i neinvazivna, lako se može integrisati u svakodnevnu kliničku praksu, jer je pokazano da je broj plućnih „kometa" bio značajno veći kod bolesnika sa SS u odnosu na bolesnike kod kojih je dispneja bila druge etiologije.

Ključne reči:
dijagnoza, diferencijalna; dispneja; srce, insuficijencija; pluća, edem; ultrasonografija.

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Introduction

With the constant demographic changes and the increase in the population age, there is a constant increase in the prevalence of heart failure (HF) in the general population, as noticed more than two decades ago [1]. The timely and correct diagnosis is of high importance for the clinicians [2]. The most commonly used methods for the assessment of the existence of HF are echocardiography and the measurements of the natriuretic peptide (NP) [3], which is a plasma biomarker of cardiac stress and HF [4]. Contrary to the traditional beliefs that the lungs are not suitable for the ultrasound (US) examination, the area of lung US is fast developing, and it provides the medical community with numerous new diagnostic opportunities [5]. Specifically, since the relationship between the alveolar interstitial syndrome and the US finding of lung "comets" (ULCs) (the so-called anterior B-lines) was described, lung US has become increasingly common. The "comets" are multiple and bilateral comet tail-like artifacts that indicate the presence of reduced air content in the lungs [5]. Lung US can also be used for monitoring the progress of illness or recovery among patients with acute HF, especially monitoring decongestion, as it is non-invasive and can be used bedside. It provides the lower necessity for repeated chest X-rays and exposure of patients to radiation. It can also be useful in the assessment of the prognosis of HF among patients, as their persistence is associated with a higher likelihood of readmission in the first six months after the initial hospitalization [6].

The aim of this study was to examine the etiology of dyspnea on admission and assess the stage of HF based on the findings of the US examination of the heart and lungs.

Methods

The cross-sectional study included a total of 110 patients treated for the symptoms of dyspnea. The study protocol was approved by the Ethics Committee of the Military Medical Academy in Belgrade (February 1, 2017). The study included all patients treated for any heart or pulmonary condition, as well as patients who reported the first episode of dyspnea without any previous illness. On admission, all patients went through a US examination of the lungs using the standard cardiological probe with a frequency of 2.5–3.5 MHz. Patients were either lying on the bed or sitting during the examination. The frontal and lateral thoracic walls between the second and fourth intercostal areas on the left from the parasternal line and between the second and the fifth intercostal areas on the right, all the way to the middle axillary line, with a total of 28 spots of examination, were examined. In all of them, we looked for the pulmonary "comets" that appear as a consequence of the increased interstitial fluid in the lungs. During the US examination of the heart, the following parameters were also examined: left ventricular end-diastolic volume, left ventricular end-systolic volume, left atrium volume, ejection fraction (EF), left ventricle (LV) diastolic function, the systolic pressure in the right ventricle, the ratio of transmitral flow velocity (E) to early diastolic mitral annulus velocity (E') (E/E'). E/E's relationship was determined using the tissue Doppler US. EF was determined using the modified Simpson method in two planes. LV diastolic function was examined using the blood flow over the mitral valve and through the pulmonary veins. The positive or pathological finding is defined as the bilateral existence of "comets" in all the anterolateral areas or lateral areas. The total score is calculated as the number of observed "comets" and reflects the grade of the pulmonary stasis [5]. At the initiation of the treatment and on discharge, patients' weight was measured, and chest X-ray imaging was done. On admission, patients' brain natriuretic peptide (BNP) level was examined as well. The additional data were taken from patient history, including age, gender, and presence of any risk factor for HF (high blood pressure, atrial fibrillation, diabetes mellitus, prior myocardial infarction, hyperlipidemia, chronic cardiomyopathy).

Statistical analyses were done using the methods of analytical and descriptive statistics. The correlation was examined using the Spearman correlation. The univariate linear regression model was used to examine the association between the examined variables and the number of "comets". According to the New York Heart Association (NYHA) classification, if the specific symptoms of HF, such as the existence of heart murmurs, edema on the extremities, and distension of neck veins, were present, chest X-ray characteristics and BNP were excluded from analysis due to co-linearity with HF. All variables that were shown to be significant were entered into the multivariate linear regression model with a number of "comets" as an outcome variable. For the determination of diagnostic accuracy of the number of "comets" for the HF as an outcome, we examined the sensitivity, specificity, and total area under the receiver operating characteristic (ROC) curve (AUC-ROC). Statistical processing and analysis were done in the SPSS version 22.0.

Results

The average age of the participants was 68.3 ± 15.5 years, and just over half (n = 58; 52.7%) of them were females.

The number of ULCs in patients with and without HF is presented in Figure 1. The mean number of registered ULCs among the total number of patients was 14.2 ± 7.4 (minimum 2, maximum 30). The mean number of ULCs among patients with HF was 18.8 ± 5.9. The mean number of ULCs among patients without HF was 8.0 ± 3.7 ($p < 0.001$).

Patients with confirmed HF had a significantly higher number of registered ULCs than patients who had symptoms of dyspnea but without confirmed HF.

The mean value of the number of ULCs in patients with normal X-ray findings was 7.5 ± 3.4, while the average number of ULCs in patients with pulmonary stasis found on X-ray imaging was significantly higher (18.0 ± 6.3). The average number of ULCs in patients with and without pulmonary stasis is presented in Figure 2.

In our study, we observed a significant difference in the total number of ULCs according to NYHA classification ($p <$

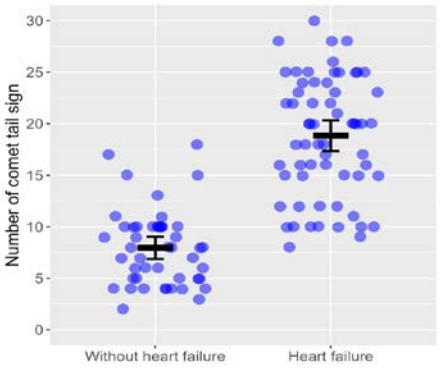

Fig. 1 – Number of ultrasound lung "comets" in relation to the presence/absence of heart failure.

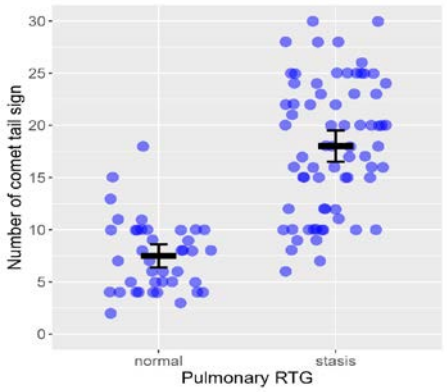

Fig. 2 – Radiography finding shows a bigger average number of ultrasound lung "comets" in relation to pulmonary stasis. RTG–X-ray.

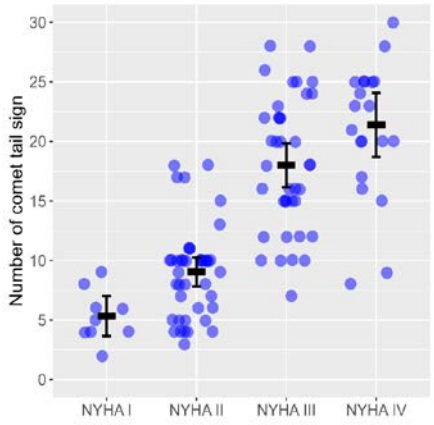

Fig. 3 – Number of ultrasound lung "comets" in patients from different classes of the New York Heart Association (NYHA) classification system.

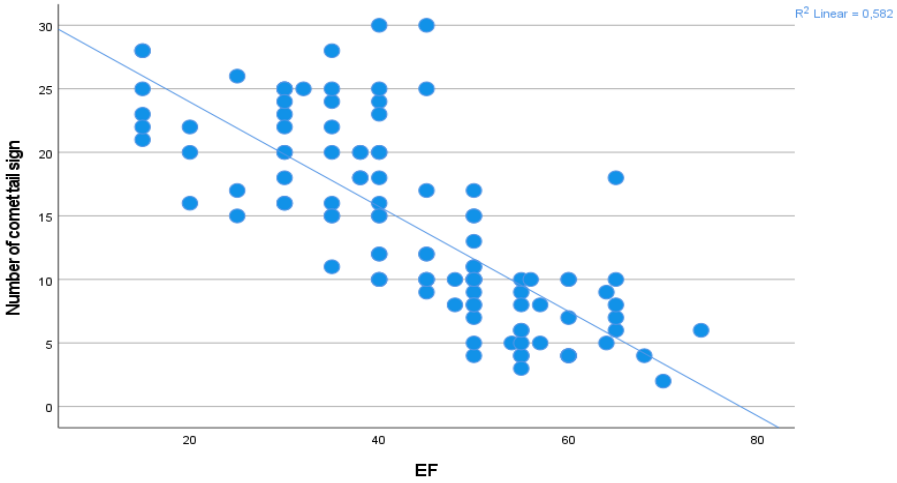

Fig. 4 – Correlation between number of ultrasound lung "comets" and ejection fraction (EF).

0.001). Patients who belonged to a higher NYHA class had a significantly higher number of registered ULCs on US examination of the lungs. Differences in the number of ULCs between patients belonging to different NYHA classes are shown in Figure 3.

There was a significant negative correlation between EF and the number of ULCs ($r = -0.80$, $p < 0.001$). Patients who had a lower EF had a significantly higher number of registered ULCs. The correlation between EF and the number of ULCs is presented in Figure 4.

We found a significant positive correlation between the number of ULCs and the BNP level (r = 0.79, $p < 0.001$); results are shown in Figure 5.

Patients who had high BNP values [median 3,912.5 pg/mL (minimum 28 pg/mL, maximum > 35,000 pg/mL); reference range < 100 pg/mL] had a significantly higher number of registered ULCs during the US examination of the lungs.

The total AUC-ROC for BNP was 96.9% (95% confidence interval: 91.8–99.3; $p < 0.001$) with BNP cut-off value at 3,805 pg/mL with high specificity (100%) and sensitivity (87.3%).

The number of ULCs had both high specificity (89.4%) and sensitivity (84.1%) for determining the HF; the optimal cut-off value for the number of ULCs for the determination of HF was 11. The AUC-ROC was 93.9% (95% confidence interval: 87.6–97.6, $p < 0.001$). The ROC curve for ULCs is presented in Figure 6.

Discussion

Lung US enables relatively quick and easy differentiation of various lung diseases, much more reliable than radiography. In addition, the examination is inexpensive and can be done next to the sick bed with a portable apparatus and repeated countless times. During the examination, the patient was spared from the application of contrast that potentially leads to kidney damage, from ionizing radiation, and from transport to distant parts of the hospital for multi-sliced computed tomography. Despite all the above advantages, US diagnostics of lung disease is still insufficiently applied, except in the diagnosis of pleural effusion. One of the possible reasons is the necessary training of doctors that lasts for months because the diagnosis depends on the quality of the image, which strongly depends on the experience of the person performing the imaging [7]. In patients with HF, fluid accumulates in the lungs, and the most characteristic sign on a US

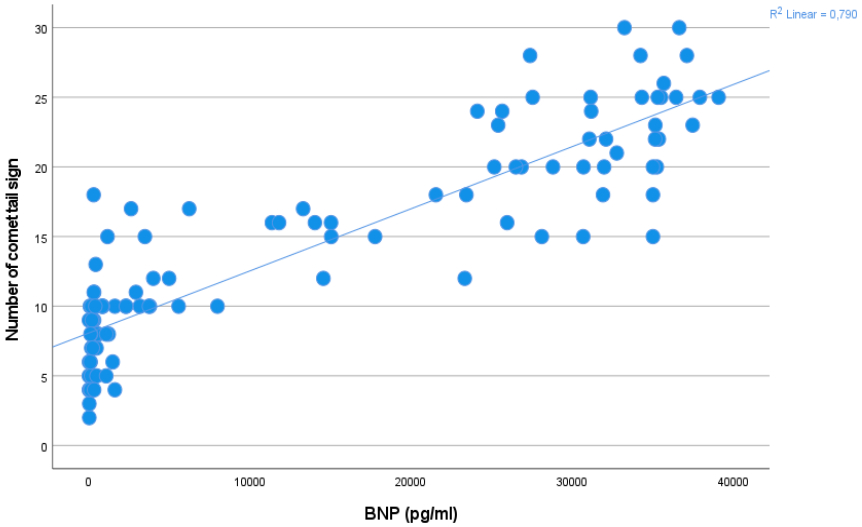

Fig. 5 – The correlation between the brain natriuretic peptide (BNP) value and the number of ultrasound lung "comets".

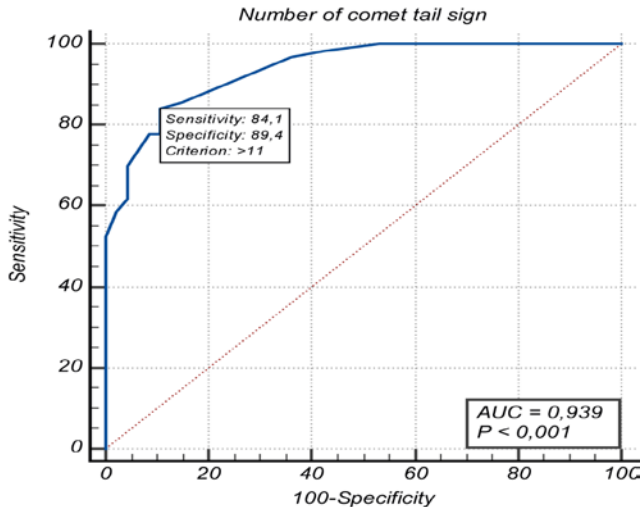

Fig. 6 – The receiver operating characteristic (ROC) curve shows the sensitivity and specificity of a number of "comets" for the prediction of heart failure (HF). AUC–area under the ROC curve.

examination of the lungs is the appearance of B-lines. These are long, vertical, hyperechoic lines that have the shape of a comet with a tail. They extend from the pleura to the inside of the lungs and move synchronously with respiration, canceling the physiological A-lines, intense and bright lines parallel to the pleura, which indicate the normal structure of the lungs [8, 9]. B-lines are the result of fluid accumulation in the interstitium, i.e., interlobular septa. They correspond to Kerley's B-lines on lung radiography in HF, i.e., horizontal lines on the periphery of the lungs, which also represent fluid accumulation in the interstitium. Ever since it was shown that recognition of multiple "comets" has high sensitivity and high specificity as a technique for evaluation of the degree of pulmonary congestion in patients with HF, the use of US as a non-invasive diagnostic tool has been established [10, 11].

There were significant differences in the number of ULCs on the US examination of heart and lung between patients with and without HF, with and without signs of pulmonary stasis on X-ray, in patients in different NYHA classes. We also found a significant negative correlation between EF and the number of ULCs. ULCs were previously shown to be markers of pulmonary congestion in patients with HF, and our results confirm the possibility of their use for diagnostic information in patients coming to the emergency department with the symptoms of dyspnea [12]. As there were significant differences in the number of ULCs according to NYHA classes, our results indicate that the ULCs may be used not only to check for the presence of HF but also as a tool to assess the grade of HF, which is also shown in previous studies [12].

We also examined the correlation between the commonly used parameter for the prediction of HF, BNP levels, and the number of ULCs, as well as its diagnostic accuracy, in order to compare the possibilities for the use of pulmonary US instead of the use of the BNP.

The measurement of the levels of BNP is used as a screening method for numerous heart diseases and commonly for assessing the progression of HF [13, 14]. The BNP levels were excluded from our final multivariate linear regression model, as there was high co-linearity with the HF, indicating a significant association between the two variables. That is in line with the results from the previous studies [15]. In our study, the total diagnostic accuracy of the number of ULCs and BNP in the prediction of HF was comparable, as the total AUC-ROC was 0.939 for the number of ULCs and 0.969 for the BNP, with BNP cut-off value at 3,805 pg/mL having the specificity of 100%. The high sensitivity of BNP (87.3 %) is in line with previous results [15], but we have also found the high specificity (100.0%), which was not reported in the Indian study [15], and the use of BNP is still a matter of disagreement [16].

Since the diagnostic accuracy of the BNP and of the ULCs appears to be comparable, there is a pronounced importance of the use of the pulmonary US assessments, especially in resource-limited settings, like low, or middle-income countries or even rural areas in developing countries in which there may not be all biochemical and molecular markers readily available. Furthermore, in some instances, the BNP seems unreliable or requires careful interpretation. These cases include racial differences, gender differences, the differences between people with different body mass index, the association of BNP levels with renal diseases, or heart diseases such as arrhythmias [17].

The study showed that when the cut-off was set at 11 ULCs, the sensitivity and specificity for the prediction of the existence of HF were 84.1% and 89.4%, respectively. That is significantly higher from, for instance, the sensitivity and specificity of Papanicolao smear for the detection of cervical cancer [18]. As the assessment of ULCs is safe and non-invasive, it can easily be integrated into daily clinical practice. Some clinicians argued that the approach we examined, using the 28 different spots for the examination of ULCs, is time-consuming and difficult for the examiner and examinee. Therefore, the new method, which is simplified and based on the seven zones compared to the previous 28 zones, was developed and was shown to correlate with diastolic functional parameters of the LV, the level of mitral regurgitation, NYHA functional classification, radiologic score, and N-terminal prohormone brain NP, all showing that this method is reliable, along with being rapid and non-invasive [10]. One study conducted in India also examined the sensitivity and specificity of the ULC assessment in the diagnosis of HF and found even higher results for both sensitivity (91%) and specificity (100%) [15]. However, the cut-off presented in the aforementioned study was 13–15 ULCs, while in our study, we used the stricter cut-off of 11 and also presented significant results regarding specificity and sensitivity.

Still, there are no developed protocols for the assessment of the quantity of pulmonary edema using the US [10]. One of the developed approaches is the so-called "28-sector" approach, which shows a linear correlation with the radiologic lung water score [10, 19, 20]. That practically means that the number of identified ULCs is correlated with the extravascular lung water examined by the X-ray or even invasive thermodilution methods.

All these findings lead to the examination of the possibility of using ULCs as a tool for diagnosis and monitoring the degree of HF for the differential diagnosis of cardiac from non-cardiac dyspnea [5].

Conclusion

The number of ULCs is significantly higher in patients with HF than in those with dyspnea of other etiologies. The number of ULCs shows a significant correlation with the clinical picture, lung radiography, NYHA class, BNP, and parameters of systolic and diastolic function of the heart. As the assessment of ULCs is safe and non-invasive, it can easily be integrated into daily clinical practice.

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Early stent thrombosis in Kounis syndrome – a case report

Kunisov sindrom i rana tromboza stenta

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Abstract

Introduction. Kounis syndrome is a simultaneous manifestation of acute coronary syndrome and conditions associated with mast cell activation, such as allergies or anaphylactic reactions. We present early stent thrombosis in a female with an atopic constitution without previous atherosclerosis of coronary arteries. **Case report.** A 50-year-old woman with typical anginal pain was admitted to the Clinic for Cardiology, University Clinical Center Niš. A few hours earlier, she had passed by a linden tree in bloom. She immediately felt chest pain, paresthesia and numbness in her left arm throat tightness, heaviness of the tongue, and swelling of the lips. The symptoms disappeared for 60 min after taking 10 mg of loratadine, but then they recurred. On the electrocardiographic (ECG) findings, 30 minutes after admission, ST elevation was seen in leads D2, D3, aVF, and V6. She underwent an emergency percutaneous coronary intervention procedure. Occlusive thrombosis was seen in the ostium of the left anterior descending (LAD) coronary artery. A sirolimus-coated stent was placed and thrombolysis in myocardial infarction (TIMI) 3 flow was obtained. A few hours after the intervention, the patient reported a new onset of chest pain followed by ventricular fibrillation (VF), DC shock, and the occurrence of new ST-segment elevation in aVR and V1–V3 on the ECG. Repeated angiography showed acute in-stent thrombotic occlusion. Balloon angioplasty was performed, which restored TIMI 3 flow in LAD coronary artery. Anticoagulant and corticosteroid therapy was administered. Three days after the intervention, optical coherence tomography imaging was performed, which showed good stent expansion and apposition, without atherosclerosis and thrombosis. **Conclusion.** Coronary angiography proved type I Kounis syndrome after exposure to an allergen, and type III Kounis syndrome developed shortly after stent placement when acute in-stent thrombosis occurred. Newly described causes of acute and subacute stent thrombosis in type III Kounis syndrome are stent-associated hypersensitivity reactions.

Key words:
angioplasty, balloon; coronary angiography; coronary disease; hypersensitivity; kounis syndrome.

Apstrakt

Uvod. Kunisov sindrom je istovremena manifestacija akutnog koronarnog sindroma i stanja udruženih sa aktivacijom mastocita, kao što su alergija i anafilaktička reakcija. Prikazujemo slučaj rane tromboze stenta kod žene sa atopijskom konstitucijom, bez prethodnog postojanja aterosklerotskih lezija. **Prikaz bolesnika.** Pedesetogodišnja žena sa tipičnim anginoznim bolom primljena je na Kliniku za kardiologiju Univerzitetskog kliničkog centra u Nišu. Nekoliko sati ranije, ona je prošla pored drveta lipe u cvetanju. Odmah je osetila bol u grudima, parestezije i utrnulost leve ruke, stezanje u grlu, otežan jezik i otok usana. Tegobe su prestale tokom 60 min nakon uzimanja 10 mg loratadina, ali su se ponovo javile. Na elektrokardiografskom (EKG) nalazu, 30 min nakon prijema, viđena je ST elevacija u odvodima D2, D3, aVF i V6. Urađena joj je perkutana koronarna intervencija. Angiogram je pokazao okluzivnu trombozu ostijalnog dela prednje silazne grane leve (*left anterior descending* – LAD) koronarne arterije. Plasiran je sirolimusom obložen stent i postignut *thrombolysis in acute myocardial infarction* – TIMI 3 protok. Nekoliko sati nakon intervencije, bolesnica je prijavila da ponovo oseća bol u grudima, što je bilo praćeno ventrikularnom fibrilacijom (VF), DC šokom i pojavom elevacije ST segmenta u aVR i V1–V3 na EKG-u. Ponovljena angiografija je pokazala akutnu *in*-stent trombozu sa okluzijom. Urađena je balon angioplastika kojom je obnovljen TIMI 3 protok u LAD koronarnoj arteriji. Primenjena je antikoagulantna i kortikosteroidna terapija. Tri dana nakon intervencije, urađena je optička koherentna tomografija, koja je pokazala dobru ekspanziju

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stenta i apoziciju, bez ateroskleroze i tromboze. **Zaključak.** Koronarografijom je dokazan tip I Kunisovog sindroma nakon ekspozicije alergenu i tip III Kunisovog sindroma ubrzo nakon postavljanja stenta, kada je nastala akutna *in*-stent tromboza. Novoopisani uzroci akutne i subakutne tromboze stenta kod Kunisovog sindroma tipa III su hipersenzitivne reakcije povezane sa stentom.

Ključne reči:
angioplastika, translumenska; angiografija koronarnih arterija; koronarna bolest; hipersenzibilnost; kunisov sindrom.

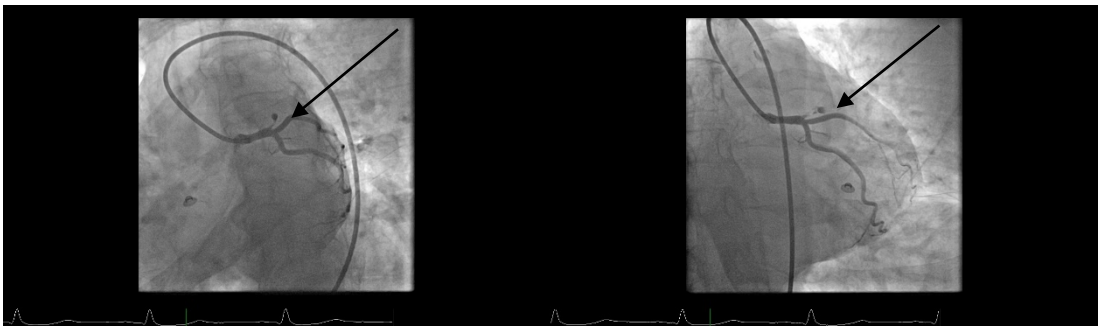
Introduction

Kounis syndrome is a simultaneous manifestation of acute coronary syndrome with conditions associated with mast cell activation, such as allergies or anaphylactic/anaphylactoid reactions [1, 2]. There are three types of Kounis syndrome. Type I represents allergic vasospasm of coronary arteries due to a release of proinflammatory mediators in patients with previously healthy coronary arteries. Type II develops when an allergic reaction occurs at the site of already present plaque in coronary arteries. Type III represents thrombotic reocclusion of epicardial coronary arteries at the site of previously treated coronary lesions. The occlusive thrombus is infiltrated by eosinophils and/or mast cells [3–6]. As Kounis syndrome is a combination of allergic and coronary-ischemic reactions, the pathogenesis, treatment, and prognosis of this syndrome are still unknown.

Case report

A 50-year-old Caucasian female patient presented to the cardiology department with typical anginal pain. A few hours earlier, she passed by a linden tree in bloom and immediately after felt chest pain, paresthesia and numbness in her left hand, throat tightness, heaviness of the tongue, and swelling of the lips. These symptoms disappeared for an hour after taking 10 mg of loratadine orally. She had a 12-year history of arterial hypertension (took ramipril 5 mg and nebivolol 5 mg once a day), long-term bronchial asthma (took vilanterol/fluticasonefluorate when needed), and allergic rhinitis (at that time without therapy). Following admission, she had an arterial blood pressure of 126/94 mm Hg, a heart rate of 69 beats/min, and an oxygen saturation of 99%. On admission, laboratory tests showed the following results: white blood cells (5.0 x 10^3/μL), neutrophils (67.4%), eosinophiles (12.8%), lactate dehydrogenase [483 U/L, reference range (RR) 0–450], C-reactive protein (1.4 mg/L, RR 0–5), creatine phosphokinase (344 U/L, RR 0–195), alanine aminotransferase (21 U/L, RR 10–42), aspartate aminotransferase (66 U/L, RR 10–37), troponin I (1.3 ng/mL, RR 0–0.4). Platelet count, kidney and liver function, as well as lipase and coagulation tests, were all within normal limits. Laboratory immunology testing results for the most common allergens were the following: IgE 665 IU/mL (normal value < 100 IU/mL); food allergy panel F1, F2, F4, F9, F13, F14 < 0.10 kU/L, FP7 0; inhalant allergy panel 8 (D1, E1, E5) was 2 kU/L (normal value < 0.10 kU/L).

On admission, 12-lead electrocardiographic (ECG) findings showed sinus rhythm, initially only with ST-segment depression in lead V1. Initial therapy included acetylsalicylic acid 300 mg, atorvastatin 40 mg, and methylprednisolone 40 mg. After 30 min, ECG showed concave ST-segment elevation in D2, D3, and aVF leads and ST elevation of 1 mm in lead V6. The echocardiographic examination showed reduced left ventricle ejection fraction (35%), remodeled left ventricle with dyskinesia of the apical segments of the inferior wall and septum. She was given a ticagrelor loading dose of 180 mg and 80 mg of low molecular weight heparin subcutaneously (sc) and underwent an emergency percutaneous coronary intervention procedure. A coronary angiogram showed occlusive thrombosis in the ostium of the left anterior descending (LAD) artery with no other changes in coronary arteries (Figure 1A and B). The lesion was treated by placing Ultimaster™ Tansei™ coronary stent 3.0 × 12 mm. The final angiogram showed thrombolysis in acute myocardial infarction (TIMI) 3 flow. We performed multiplate analysis as a standard for all patients, and the result showed adequate response to acetylsalicylic acid and ticagrelor. A few hours following the intervention, the patient reported a new onset of chest pain followed by ECG signs of ST-segment elevation in aVR, V1–V3 leads. Another coronary angiography was performed and showed an acute in-stent occlusion with a thrombotic mass followed by a small



A) **B)**

Fig. 1 A) and B) – Angiography following admission – left anterior descending coronary artery occluded

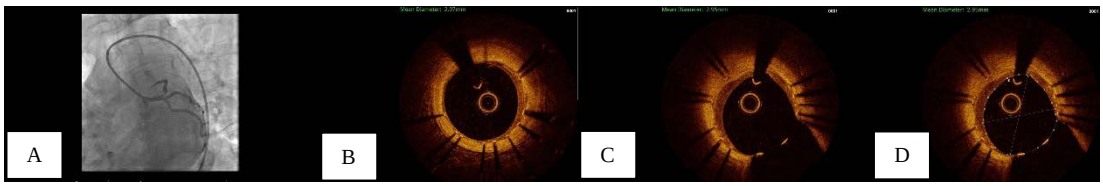


Fig. 2 – Angiography after repeated ST-segment elevation: A) in-stent thrombosis. Optical coherence tomography after second percutaneous coronary intervention procedure: B) optimal stent expansion and apposition; C) no residual stenosis; D) no stent fracture.

thrombus in the left circumflex artery (Figure 2A). Balloon angioplasty was done, which restored TIMI 3 flow in the LAD artery. Anticoagulant and corticosteroid therapy was administered (enoxaparin 60 mg/12 hrs s.c., dexamethasone 4 mg/24 hrs i.v.) beside atorvastatin 80 mg and dual antiplatelet therapy for seven days. Due to the simultaneous onset of the symptoms of allergic reaction and acute myocardial infarction, as well as acute stent thrombosis, intravascular imaging was performed. Three days following the intervention, optical coherence tomography (OCT) imaging showed fully structurally preserved arterial walls without any signs of atherosclerosis, no signs of restenosis, adequate stent expansion, and good apposition. An organized thrombus was present, occupying up to 10% of the artery lumen (Figure 2B–D).

At hospital discharge, our patient received acetylsalicylic acid 100 mg, ticagrelor 90 mg twice daily, atorvastatin 40 mg, zofenopril 15 mg, spironolactone 25 mg, and nebivolol 1.25 mg.

Prior to submitting this case report to the journal, the patient gave written informed consent.

Discussion

Kounis syndrome is not a rare disease; the incidence is approximately 0.1%, but it is most often left undiagnosed. It affects all ages, races, and both genders and could be fatal [7].

There are three types of Kounis syndrome based on the cause of cardiogenic involvement. Type I represents an allergic vasospasm of coronary arteries due to a release of proinflammatory mediators in patients with previously healthy coronary arteries, leading only to endothelial dysfunction. Manifestations of this variant start from transitory coronary artery spasm without a significant increase in cardiac biomarkers to the development of acute myocardial infarction with clear ECG changes, wall motion abnormalities, and a rise in cardiac biomarkers. This type is considered to be one of the causes of myocardial infarction without occlusion of the coronary artery - MINOCA. Type II develops when an allergic reaction occurs at the site of already present plaque in coronary arteries. The release of proinflammatory mediators from mastocytes, eosinophils, and basophils leads to vasospasm, destabilization, erosion, and rupture of atherosclerotic plaque with the development of acute myocardial infarction. Type III represents thrombotic reocclusion of previously treated coronary lesions (stent thrombosis or thrombosis after percutaneous balloon angioplasty – POBA) [1–6].

For the diagnosis of Kounis syndrome, there are no specific criteria. Its presentation could vary from cardiac arrhythmias to sudden cardiac death with different ECG and laboratory changes [8].

In the diagnosis of Kounis syndrome, the first step is to determine the history of allergy reactions, which we found in our patient. The allergic reaction to the trigger is known in 25% of patients with this syndrome. We did find high levels of IgE, but its role is not clearly elucidated in diagnosis. Serum levels of histamine and tryptase could be helpful but are released quickly after contact with the allergen and quickly removed from the circulation (up to 90 min for tryptase and even shorter for histamine). In 60% of all patients with this syndrome, the troponin levels are elevated, which was also the case with our patient [9].

ECG changes on admission and echocardiography findings do not correlate with the angiography showing a thrombus at the ostium of LAD artery. The inferior and lateral wall involvement could be related to coronary vasospasm and/or coronary embolism and endothelial dysfunction. Coronary arteries are not the direct target organ of hypersensitivity, and it could be hypothesized that the vasospasm might be secondary to the smooth muscle contraction reflex caused by the irritation of the bronchial epithelium by allergens, among other known mechanisms. Presumably, the bronchial smooth muscle contraction reflex induces the epithelium-derived inflammatory molecules accumulation and could cause coronary vasospasm through this pathway [10].

Our paper describes early stent thrombosis in a female patient with an atopic constitution without previous atherosclerotic lesions of coronary arteries. Serologic analyses showed high IgE levels for common inhalant allergens, and the patient has a history of bronchial asthma. Coronary angiography showed type I Kounis syndrome after exposure to an allergen, and shortly after stent placement, type III Kounis syndrome developed.

Local and systemic factors lead to early stent thrombosis. Insufficient stent expansion, stent malposition, and tortuosity of the vessel are the most common local factors. Systemic factors include hypercoagulable conditions, malignancy, and use of chemotherapeutics [4, 11]. We performed the OCT to ensure that local factors were not the cause of early stent thrombosis.

Newly described causes of acute and subacute stent thrombosis in type III Kounis syndrome are stent-associated hypersensitivity reactions [11, 12]. Surface IgE receptors on subtypes of platelets are thought to be associated with the initia-

tion of stent thrombosis in reactions of hypersensitivity. The platelets with IgG receptors (FCγRII) and IgE receptors of high and low affinity (FcεRI and FCεRII) is responsible for the activation of prothrombotic events cascade in reactions of hypersensitivity. During this activation, platelets produce proinflammatory (platelet-derived factor 4, platelet growth factor, CD154), procoagulant (factor V, factor XI, plasminogen activator inhibitor 1), adhesive (thrombospondin, fibrinogen, p-selectin, Von Wilebrand factor), and chemotactic (adenosine diphosphate, adenosine triphosphate, serotonin, histamine, calcium, and magnesium ions) mediators which propagate, enhance, and maintain the process of thrombus formation. That leads to conformational changes in the GP IIb/IIIa receptor and enables fibrinogen to attach platelets and further aggregation [11, 13, 14].

Our patient had stent thrombosis after the implantation of Ultimaster™ Tansei™ stent (Terumo, Tokyo, Japan) with cobalt-chromium structure and poly-(d,l-lactic) acid and poly-(l-lactide-co-ε-caprolactone) and sirolimus-impregnated secondary coating. As studies have shown, sirolimus has an inhibitory role in eosinophilic infiltration and histamine synthesis; therefore, there is a small chance for the development of hypersensitive reactions [15, 16]. Ultimaster™ Tansei™ stent, applied in this patient, has not been associated with reactions of stent thrombosis up to now. A case of a female patient with repetitive thrombosis was described by Jimba et al. [17], however, an adverse reaction was not as severe as was described in our patient. Acute stent thrombosis associated with hypersensitive reaction to the stent itself was described only in a few papers [15, 18]. A small number of reported cases can be explained with unclear clinical presentation, atypically manifested atopic reaction, demanding diagnostics that could involve thrombus aspiration, pathohystological diagnostics, and use of intravascular imaging. Yamaji et al. [19] found a significantly higher eosinophilic cell fraction in patients with very late in-stent thrombosis (> 12 months after intervention) compared to those with early (up to one month after intervention) and late (1 to 12 months after intervention) in-stent thrombosis. A probable cause of this finding is hypersensitive vasculitis [20].

The etiology of stent thrombosis in our patient could be a prolonged allergic reaction or hypersensitivity to stent components.

Management of Kounis syndrome is complex, and we do not have established recommendations. Two goals in the treatment of Kounis syndrome are to dilate coronary arteries and manage an allergic reaction. Therefore, patients with Kounis syndrome are initially treated with steroids, antihistamines, fluid resuscitation, epinephrine, oxygen, and antithrombotics. Subsequently, in Kounis syndrome type II, the standard therapy for acute coronary syndrome should be given. Since the coronary vasospasm is dominant in young and previously healthy patients, they should receive nitrates and calcium-channel blockers. Beta-blockers should be administrated very carefully in the acute phase since they can interfere with the epinephrine and induce or aggravate the spasm of coronary arteries by leaving alpha-adrenergic receptors unblocked [21, 22]. Beta-blockers may even increase the production and release of anaphylaxis mediators. Furthermore, morphine, which is important in the treatment of acute chest pain, should be avoided in Kounis syndrome since it could induce histamine release and further aggravate the allergic reaction. Paradoxically, epinephrine, which is routinely used in anaphylaxis, may worsen coronary vasospasm and aggravate ischemia, so active monitoring is needed during its administration [23, 24]. After the acute phase, standard therapy should be given. Our patient received a beta-blocker at hospital discharge and standard therapy for acute coronary syndrome, including the aldosterone receptor antagonist for left ventricular dysfunction.

Conclusion

The factors involved in the propagation of the hypersensitivity cascade in this patient were involved in the development of acute stent thrombosis. What makes this patient special compared to previously published cases is the absence of atherosclerosis at the site of a culprit lesion. OCT demonstrated only the presence of a thrombus but not the underlying plaque. Analyzing the cases published so far and taking into consideration all the circumstances in this case, special attention should be paid to repeated occlusion of the culprit artery in the further course of healthcare. Fear of recurrence of thrombosis and occurrence of restenosis with the existing association of atopy, inflammation, and thrombosis is justified. The contributing factors are both systemic hypersensitivity reaction and local effect of the applied coronary stent, with the release of potential allergens such as metal anions, products of polymer decomposition, and drugs, all of which are the active substances that could lead to the occurrence of very late in-stent thrombosis.

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Infantile hemangioma of the upper eyelid in one very and two extremely preterm infants

Infantilni hemangiom gornjeg kapka kod jednog veoma prevremeno i dva ekstremno prevremeno rođena odojčeta

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Abstract

Introduction. Infantile hemangiomas (IHs) are the most common vascular tumors of infancy whose frequency increases with decreasing gestational age. Although rare, periorbital IHs (PIHs) have a high complication rate, with a substantial risk of impaired vision and aesthetic consequences. Because they are considered high-risk IH, such patients must be treated. In the available literature, there is little information about the treatment of IHs in very and extremely preterm newborns and infants. **Case report**. We present three male infants (one very and two extremely prematurely born) with PIHs involving the upper eyelid. In all three cases, IHs were solitary but with different subtypes according to soft-tissue depth (superficial, combined, deep). No additional congenital anomalies were found in any of these cases. An individualized approach to the management of each patient was applied. All infants were treated with oral administration of propranolol, with careful monitoring for potential side effects of the drug and adjustment of treatment, following their eventual occurrence. The introduction of oral propranolol was carried out in a hospital setting, with a gradual increase of the drug dose until the target dose was reached. **Conclusion.** In the case of PIH, an ophthalmologist is an inevitable part of the medical team. Very and extremely preterm infants are already under the supervision of an ophthalmologist due to mandatory screening for retinopathy of prematurity; however, if they also have PIHs, monitoring must be particularly detailed and long-lasting.

Key words:
drug therapy; eyelids; hemangioma; infant, extremely premature; infant, premature; propranolol.

Apstrakt

Uvod. Infantilni hemangiomi (IH) su najčešći vaskularni tumori u periodu odojčeta, čija se učestalost povećava sa smanjenjem gestacijskog doba. Mada retki, periorbitalni IH (PIH) imaju visoku učestalost komplikacija, sa značajnim rizikom od oštećenja vida i estetskih posledica. Zbog toga što se smatraju visokorizičnim IH, takvi bolesnici moraju biti lečeni. U dostupnoj literaturi ima malo podataka o terapiji IH kod novorođenčadi i odojčadi rođene sa veoma niskom i ekstremno niskom gestacijom. **Prikaz bolesnika**. Prikazujemo troje odojčadi muškog pola (jedno rođeno veoma prevremeno a dvoje ekstremno prevremeno) sa PIH koji je zahvatio gornji kapak. U sva tri slučaja, IH su bili pojedinačni, ali sa različitim podtipovima prema dubini zahvatanja mekog tkiva (površinski, kombinovani, duboki). Nijedan od prikazanih bolesnika nije imao dodatne kongenitalne anomalije. Za lečenje svakog bolesnika primenjen je individualni pristup. Sva odojčad su lečena oralnom primenom propranolola, uz pažljivo praćenje mogućih neželjenih efekata leka i prilagođavanje lečenja, u skladu sa njihovom eventualnom pojavom. Uvođenje propranolola *per os* sprovedeno je u bolničkim uslovima, sa postepenim povećavanjem doze leka do postizanja ciljne terapijske doze. **Zaključak.** U slučaju PIH, oftalmolog je neizostavni član medicinskog tima. Veoma nezrela i ekstremno nezrela novorođenčad su pod nadzorom oftalmologa u sklopu obaveznog skrininga na retinopatiju, ali, ukoliko imaju i PIH, nadzor mora biti posebno detaljan i dugotrajan.

Ključne reči:
lečenje lekovima; kapak; hemangiom; novorođenče, prevremeno, ekstremno; novorođenče, prevremeno; propranolol.

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Introduction

Infantile hemangiomas (IHs) are the most common vascular tumors of infancy, which occur in as many as 5% of infants. They are benign vascular endothelial neoplasms that usually appear during the first weeks of life and go on to enter a proliferative phase, after which they show spontaneous regression – involution phase [1]. During the proliferative phase, which usually lasts from 6 to 12 months, numerous complications can emerge. The clinical significance of these complications is largely determined by the localization and type of IH, but some characteristics of the patient can also play an important role. We present three clinical cases of very preterm [born from 28 to 32 gestational weeks (GW)] and extremely preterm (born with less than 28 GW) infants with a solitary IH involving the upper eyelid. Through these case reports, we would like to point out the clinical characteristics and the approach to IHs of this particular localization and this specific group of infants.

Case report

All three presented infants were diagnosed, treated, and followed up in a single regional tertiary-care university hospital. Data on their prenatal history, birth, primary hospitalization, and the appearance, characteristics, therapy, and follow-up examinations of their IHs was retrospectively collected from their medical records, as well as from a photo archives database.

In all three cases, IHs were solitary without any accompanying anomalies, which was confirmed by repeated, detailed physical examinations, ultrasound of the brain and abdominal organs, as well as echocardiography. The diagnosis of IH, in all three cases, was established based on a typical clinical finding, aided by a Doppler ultrasound examination in the third patient shown.

The main perinatal data, IH characteristics, and therapy in all three clinical cases are shown in Table 1.

Case 1

In this case, we present a very preterm male infant, born at 30 GW with a birth weight of 1,570 g from a second spontaneously conceived pregnancy. He was born by an emergency cesarean section due to an acute illness of the mother. The infant suffered severe asphyxia at birth, requiring supplemental oxygen and noninvasive respiratory support. He was treated for several diseases associated with prematurity: respiratory distress syndrome (RDS), neonatal jaundice, late-onset sepsis, meningitis, and anemia of prematurity. Furthermore, regular screening for retinopathy of prematurity (ROP) revealed stage 1 ROP, with no need for intervention, and with the regression of changes on the retina at subsequent follow-ups.

At the postnatal age of 2 weeks, the infant developed an IH on his left upper eyelid. It was flat, with bright red papules on an erythematous background. There were also vasoconstriction patches and fine telangiectasias (Figure 1A).

During the next several weeks, the IH did not change in size. It was still at the level of the surrounding skin with an erythematous background, which was more pronounced while telangiectasias became coarser. Based on the evolution of the IH and its clinical appearance, it was classified as a superficial IH with minimal or arrested growth (MAG) – IH-MAG. At the age of 74 postnatal days, a nonselective beta-blocker – propranolol, was introduced in the hospital setting, accompanied by careful monitoring for potential adverse effects (AE) of the therapy. Propranolol was administered orally with a gradual increase of the dose to a therapeutic dose of 2 mg/kg divided into three equal daily doses. After reaching the target dose of propranolol, the infant was monitored on an outpatient basis, with the periodic dose adjustment of propranolol according to the infant's weight gain. Slow and subtle but progressive regression of IH was observed. At the postnatal age of six months, IH-MAG was paler and flat with no signs of ulceration (Figure 1B). On subsequent follow-up exams, further regression was observed with the continued propranolol therapy. No ophthalmological complications have been noticed so far.

Table 1

Infants' main perinatal data and clinical characteristics and therapy course of the upper eyelid infantile hemangiomas

Parameter	Case 1	Case 2	Case 3
Gestation (GW)	30	27 $^{5/7}$	27 $^{6/7}$
Birth weight (g)	1,570	1,010	1,150
Gender	male	male	male
Single-twin-triplet	single	triplet	twin
Mode of conception	spontaneous	IVF	IVF
Postnatal age (days, months/CGW) at the time of IH onset	15 days/32 GW	2.5 months/38 GW	21 day/30 $^{6/7}$ GW
Type of IH	superficial	combined	deep
Visual axis obstruction by the IH	no	yes	yes
Postnatal (days) and CGW/CA) of initiation of oral propranolol therapy	74 days/40 CGW	160 days/10 weeks CA	1st 66 days/ 37 $^{3/7}$ CGW 2nd 82 days/ 39 $^{4/7}$ CGW
Complications of oral propranolol therapy	none	none	apnea, hypoglycemia

GW – gestational weeks; IVF – *in vitro* fertilization; CGW – corrected gestational weeks; IH – infantile hemangioma; CA – corrected age.

Fig. 1 – Patient 1 with the infantile hemangioma (IH) with minimal or arrested growth (MAG) – IH-MAG, on the left upper eyelid: A – at the postnatal age of 1 month, before propranolol therapy: IH appears as flat, with bright red papules on an erythematous background. Vasoconstriction patches and fine telangiectasias were also present; B – at the postnatal age of 6 months, after 3.5 months of propranolol therapy: IH-MAG was paler and flat with no signs of ulceration.

Case 2

In the second case, we present an extremely preterm male infant born at 27 $^{5/7}$ GW with a birth weight of 1,010 g as the second triplet from the first trigeminal pregnancy conceived through *in vitro* fertilization (IVF). He was born by an emergency cesarean section due to preterm contractions and the onset of spontaneous labor. The infant suffered severe asphyxia at birth, demanding resuscitation, endotracheal surfactant, and mechanical ventilation. He was treated for several prematurity-related complications: RDS, bronchopulmonary dysplasia (BPD), anemia of prematurity, late-onset sepsis, and intracranial hemorrhage grade I. Moreover, he suffered from a severe form of ROP treated with intravitreal application of vascular endothelial growth factor antagonist (anti-VEGF).

After he was discharged, at the postnatal age of about 2.5 months, his parents noticed a solitary red elevated lesion located on the right upper eyelid. At the postnatal age of 4.5 months, his IH was examined at our clinic for the first time during regular follow-up of the infant's neurodevelopment. At that point, an ill-defined, oval purple mass in the inner corner of the right upper eyelid was noted. The edge of the upper eyelid was deformed, and partial visual axis obstruction was evident (Figure 2). Since there were both superficial and deep components, the IH was classified as a combined IH.

Considering the interference with the visual axis, the infant was hospitalized in order to introduce oral propranolol therapy. The dose was gradually increased, reaching the target dose of 2 mg/kg, divided into three daily doses. Thereafter, the infant was monitored on an outpatient basis, with the periodic dose adjustment of propranolol. Very soon after the introduction of propranolol, a significant reduction of the IH was observed with complete regression after only 12 weeks of therapy. At the postnatal age of nine months, propranolol

Fig. 2 – Patient 2 with combined infantile hemangioma of the inner corner of the right upper eyelid at the postnatal age of 4.5 months (before propranolol therapy): oval purple mass in the inner corner of the right upper eyelid was noted; the edge of the upper eyelid was deformed, and partial visual axis obstruction was evident.

was gradually discontinued. On subsequent follow-up exams, no IH rebound was noticed. Ophthalmological follow-up examinations did not show significant visual complications until the age of 15 months.

Case 3

In the third case, we present a male extremely preterm infant, the first-born twin conceived through IVF. He was born at 27 $^{6/7}$ GW by an emergency cesarean section due to eclampsia of the mother, with a birth weight of 1,150 g. At birth, the infant suffered severe asphyxia and RDS, demanding surfactant therapy and respiratory support. In the further course, besides late-onset sepsis and anemia of prematurity, he showed prolonged supplement oxygen dependency due to a severe form of BPD, treated with systemic corticosteroid therapy. In addition, he was diagnosed with severe ROP demanding treatment (intravitreal application of anti-VEGF).

At that time, an IH of the right upper eyelid was already present. It emerged at the postnatal age of 21 days in the form of large light blue–purple swelling covering the entire upper eyelid. Overlaying skin was intact, and fine telangiectasias were present. The IH was classified as a deep IH, and a decision on an active approach was made. Due to the previously mentioned complications of prematurity related to respiratory functions, systemic therapy of the IH with oral propranolol was postponed. In the meantime, the IH grew until it completely obstructed the visual axis, which was accompanied by excessive tearing (Figure 3). At the age of 66 postnatal days, during the primary hospitalization, the infant did not need supplemental oxygen and oral propranolol was introduced, starting with a dose of 0.5 mg/kg. However, on the second day of propranolol administration, apnea and hypoglycemia occurred, and propranolol therapy was discontinued. Following the stabilization of respiratory and metabolic functions, on the 82nd postnatal day (39 $^{4/7}$ corrected GW), another attempt was made to administer propranolol. The absence of previously noted AE enabled the gradual increase of the dose to the target therapeutic daily dose of 2 mg/kg divided into three equal daily doses. Soon after reaching the target dose of propranolol, the visual axis was partially opened. After discharge, the infant was carefully monitored on an outpatient basis, with the periodic dose adjustment of propranolol. Slow IH regression was noticed, almost completely freeing the visual axis. For precise monitoring of the regression of the deep portion of the IH, a magnetic resonance scan of the endocranium was performed after 12 months of propranolol therapy (at the postnatal age of 15 months). It revealed a significant reduction in volume of the IH of the right orbit and eyelid, with a minor retention in the cranial aspect of the orbital compartment. At the postnatal age of 17 months, IH of the right upper eyelid was still present, with consecutive discrete asymmetry of the *rima oculi*. Strabismus was noticed on ophthalmic follow-up visits. Visual evoked potential testing showed no significant deviation for age or asymmetry of the left and right eye.

Discussion

IHs are mostly found within the head and neck region, but the periocular area itself is rarely affected. In a population-based study, Alniemi et al. [2] calculated the occurrence rate of periocular IHs as 1 in 1,586 live births, with the IH being most prevalently located unilaterally on the upper eyelid. All three of our infants presented with an IH of the upper eyelid.

Fig. 3 – Patient 3 with deep infantile hemangioma (IH) of the right upper eyelid at the postnatal age of 2 months (before propranolol therapy): IH completely obstructed the visual axis, accompanied by excessive tearing.

The clinical significance of an IH is largely reflected by its localization as well as by its type regarding the anatomical spread and the depth of the affected tissue. Depending on the anatomic appearance, IHs may be categorized into four patterns: localized/focal (well-defined focal lesions), segmental (involving an anatomic region, often plaque-like and > 5 cm in diameter), indeterminate/undetermined (neither localized nor segmental), and multifocal (multiple discrete IHs at different body sites). IHs may also be classified with regard to soft-tissue depth as superficial (localized within the skin, with no evident subcutaneous component – appearing red in color), deep (located below the skin's surface, lower dermis or subcutis – appearing blue), combined/mixed (having both a superficial and deep component) [1]. A special subtype of superficial IH is referred to as IH-MAG, named after an important clinical characteristic – lack of an obvious proliferative phase [3, 4]. All of the presented infants had a localized or focal IH (ranging from 1–3 cm in maximal size) but with different subtypes according to soft-tissue depth, showing that all types of IH could be found on a small anatomic region.

Periorbital IHs (PIHs) have a high complication rate of 63%, which is nearly three times higher than that of the IHs located at other sites on the body [5].

Potential cutaneous sequels of high-risk IHs include anetodermic (outpouching) skin, redundant skin, and scarring (after ulceration) [6]. The incidence of ulceration of the IH surface varies from 5% to 21% and most commonly occurs in infants younger than four months, with preterm birth increasing the risk. IHs that are large, superficial or mixed, and/or segmental are more predisposed to ulceration [6]. The consequence of ulceration, besides pain, bleeding, or potential for a secondary infection, is the formation of a permanent scar. In addition to the aesthetic consequences of a scar on the eyelid, there is considerable risk for an incomplete closure of the eyelid due to the scar, and this, in turn, disrupts the function of the eye.

Furthermore, IHs on the eyelid pose a risk for vision impairment. Ocular complications such as ptosis, strabismus, anisometropia, astigmatism, and even amblyopia are not so rare in IHs involving the periocular region [1, 7]. During the infants' visually sensitive period, hemilateral visual deprivation leads to vision impairment, called "stimulus-deprivation amblyopia (SDA)" [8]. SDA can occur through direct pressure by the tissue mass of the hemangioma on the globe (causing astigmatism or myopia), by occlusion of the visual axis, or induction of strabismus due to mass effect [9]. Features of IHs of the upper eyelid associated with worsened visual outcomes are IHs greater than one centimeter, ptosis, proptosis, globe displacement, strabismus, and occlusion [9, 10]. Some IHs (mixed and deep IHs) may cause exophthalmos that can lead to exposure keratopathy and tear duct obstruction [9]. In preterm infants, especially those born with very and extremely small gestational age, vision function is already significantly impaired by prematurity. It is well known that premature infants, especially those with ROP, are at a significantly increased risk of a number of disorders of the eye and vision function, such as strabismus, myopia of prematurity, reduction of visual acuity, visual field deficits, deficits in contrast sensitivity, and vision loss [11]. An additional threat to such an important function for the quality of life as vision is the existence of a periocular IH, which is particularly burdensome in this subpopulation. Very and extremely preterm infants are already under the supervision of an ophthalmologist because of the need for ROP screening. However, if there is an additional risk factor for visual function, such as an IH of the eyelid, ophthalmologic monitoring must be particularly detailed and long-lasting since some visual complications can be confirmed only at a more advanced age.

Most low-risk, small, solitary IHs do not require any treatment. These low-risk IHs should be managed through an active non-intervention follow-up, with an emphasis on parental education on the nature of the IH [12].

According to the recently published Clinical Practice Guideline for the Management of Infantile Hemangiomas [1], those referred to as high-risk (HR) IHs require systemic treatment. If there is evidence or potential for IH to cause life-threatening complications, functional impairment or ulceration, structural anomalies, or permanent disfigurement, it should be classified and treated as an HR IH. Based on these criteria, the IH of the eyelid is certainly in an HR group and should be managed accordingly.

The first-line drug for HR IHs is orally administered propranolol [1], at a target dose of 2 to 3 mg/kg *per* day. With reports of up to 98% of IH responding favorably to treatment, propranolol is stated to be highly effective [6]. For all three cases, there was a clear indication for systemic therapy, bearing in mind the diameter greater than 1 cm, localization on the upper eyelid, and the burden of ocular complications.

Although propranolol is usually administered continuously for at least six months and maintained until 12 months of age [1], the duration of treatment is determined by a variety of factors, including age, hemangioma location, hemangioma subtype, and age at initiation of therapy [6]. Individualized approach is illustrated with the second presented infant, in whom propranolol therapy was withdrawn after only five months, with complete regression of IH and without IH rebound on long-term follow-up.

Propranolol has been proven to be very effective and safe for IH therapy [13, 14]. However, like any medicine, propranolol can cause side effects, among which clinically most important are hypoglycemia, hypotension, bradycardia, and bronchospasm [1]. These side effects are considered to be more frequent and more intense in such a specific and vulnerable group of patients as very and extremely preterm infants.

There is little information about the treatment of IHs (in general, as well as IHs of the periocular region) in very and extremely preterm infants. Most recommendations can be deduced from published papers and clinical experiences on term-born infants. The aforementioned guideline might be viewed as a stronghold for further investigation and trials in the pursuit of adequate treatment of this particular patient population, which is generally under a higher risk for the development of IHs, complicated IH course (ocular complications and ulceration), and increased risk of side-effects of the therapy. Very and extremely preterm infants are, in many aspects, especially demanding in the management of many

medical problems, IH of the eyelid included. The third infant in our case series showed AE of propranolol during the gradual increase of the dose, so it was temporarily discontinued to be administered at a later age. In this way, his eyelid IH grew, and his eye was occluded for a longer period than would be the case with the periocular IH of the same starting characteristics, appearing in a term infant at a later postnatal age.

Conclusion

IH on the eyelid must be considered high-risk and treated with systemic therapy as soon as possible. Significant risk of ulceration with consecutive scarring, eyelid deformity, and aesthetic consequences, as well as the possibility of a negative effect on vision function, requires treatment with the fastest and most complete effect; this effect can be achieved by oral beta-blocker therapy. The introduction of oral propranolol in preterm infants, especially those born very and extremely preterm, is best performed in a hospital setting, with gradual achievement of the target dose of the drug and careful monitoring of possible side effects of the therapy. An ophthalmologist is a mandatory part of the medical team for the follow-up of infants and children with infantile hemangioma on the eyelid, especially if they are preterm.

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CASE REPORT

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Organ preservation surgery for laryngeal cancer in a trombone player

Funkcionalna hirurgija karcinoma larinksa kod tromboniste

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Abstract

Introduction. Modern treatments for early glottic carcinoma achieve high rates of local control and long-term survival, but potential side effects of some of these treatments have a substantial impact on a patient's quality of life. There is a small quantity of available scientific research on the effects of organ-preservation surgery on musicians, highlighting the challenge of balancing functional outcomes with their occupational demands. **Case report.** We present a successful surgical treatment of a mid-membranous left vocal fold squamous cell carcinoma (T1a stage) in a professional trombonist with a history of many years of smoking. Due to suboptimal exposure during initial microlaryngoscopy, open cordectomy was performed for tumor removal instead of transoral laser microsurgery. After the operation and the proper rehabilitation, the patient continued to play the brass instrument unhindered and managed to fulfill all the obligations of a professional musician in a national orchestra. **Conclusion.** Selecting a method for the treatment of glottic cancers in professional musicians who play brass instruments remains challenging due to limited literature and the potential harm to the ability of performance and the musician's career. Partial open laryngectomies for laryngeal cancer treatment are shown to be feasible without compromising the musician's performance.

Key words:
laryngeal neoplasms; laryngectomy; quality of life; carcinoma, squamous cell; vocal cords.

Apstrakt

Uvod. Savremenim tretmanima karcinoma glotisa, otkrivenog u ranoj fazi, postiže se visoka stopa lokalne kontrole bolesti i dugoročno preživljavanje, ali potencijalni neželjeni efekti nekih tretmana mogu imati značajan uticaj na kvalitet života bolesnika. Malo je dostupnih naučnih istraživanja o efektima poštedne hirurgije kod muzičara, pri čemu je naglašen izazov balansiranja između funkcionalnog ishoda hirurgije i njihovih profesionalnih zahteva. **Prikaz bolesnika.** Predstavljamo uspešno hirurško lečenje skvamocelularnog karcinoma (T1a stadijum) srednje trećine levog glasnog nabora kod profesionalnog tromboniste sa višegodišnjom istorijom pušenja. Zbog suboptimalne ekspozicije tokom inicijalne mikrolaringoskopije, za uklanjanje karcinoma urađena je otvorena hordektomija umesto transoralne laserske mikrohirurgije. Nakon operacije i pravilne rehabilitacije, bolesnik je nastavio da nesmetano svira duvački instrument i uspeo da ispuni sve obaveze jednog profesionalnog muzičara u nacionalnom orkestru. **Zaključak.** Izbor metode lečenja od karcinoma glotisa kod profesionalnih muzičara koji sviraju duvačke instrumente, ostaje izazov zbog ograničenih literaturnih podataka i potencijalne štete za profesionalnu sposobnost i karijeru muzičara. Pokazalo se da su delimične otvorene laringektomije za lečenje od karcinoma larinksa izvodljive bez ugrožavanja performansi muzičara.

Ključne reči:
larinks, neoplazme; laringektomija; kvalitet života; karcinom, planocelularni; glasne žice.

Introduction

Modern treatments for early glottic carcinoma (tumor *in situ*, T1 stage) include local radiotherapy (RT), transoral laser microsurgery (TLM), and open cordectomy or partial laryngectomy. Each treatment method achieves a high rate of local control and long-term survival [1]. Although the oncologic and functional outcomes are comparable, side effects may vary greatly and have a substantial impact on the quality of life of the patient [2]. Playing a brass wind instrument necessitates not just the patient's upper airway but also the functioning larynx to efficiently conduct, regulate, and modulate the airstream required for tone creation [3]. There is a lack of research on the effects of organ-preservation therapy for laryn-

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geal carcinoma in musicians who play wind instruments. We describe a case of a professional trombonist diagnosed with a T1a left vocal fold squamous cell carcinoma (SCC), treated with open cordectomy. According to our knowledge, this is the first documented example of an orchestral musician resuming the same level of professional activities following oncological larynx preservation surgery.

Case report

A 55-year-old male patient with an otherwise unremarkable medical history was evaluated for long-standing hoarseness and subsequently diagnosed with a T1a keratinizing SCC of the mid-membranous left vocal fold. He had a 24 pack-year smoking history before quitting 14 years ago. Apart from his medical history, he was a professional trombonist, playing various opera and ballet repertoire as a full-time member of a major theater orchestra. The case was presented to the multidisciplinary tumor board, after which the patient decided to proceed with the surgical intervention. All therapy choices and their prospective effects on the patient's health and career were thoroughly reviewed with him before a final decision was made. Due to the suboptimal exposure of the glottis during the microlaryngoscopy, left-sided open cordectomy in general anesthesia was performed instead of the usual TLM. The thyroid cartilage was approached through the small vertical incision of the skin and subcutaneous tissues of the anterior neck. Following division and retraction of the infrahyoid muscles in the midline, the left vocal fold with the ipsilateral vocal process of the arytenoid was removed in an "en-bloc" fashion through laryngofissure. The resulting defect was left to epithelialize, and the incision was closed in layers. The final histopathology report confirmed the initial diagnosis, with complete tumor removal and tumor-free margins of the vocal fold specimen. The postoperative period was uneventful, and the patient was discharged on the seventh postoperative day, with regular monthly follow-ups by the attending surgeon (Figure 1). Two months after surgery, the patient began breathing exercises and mouthpiece buzzing without the instrument, and four months after surgery, he gradually resumed performances in the orchestra (Figure 2) (Video 1).

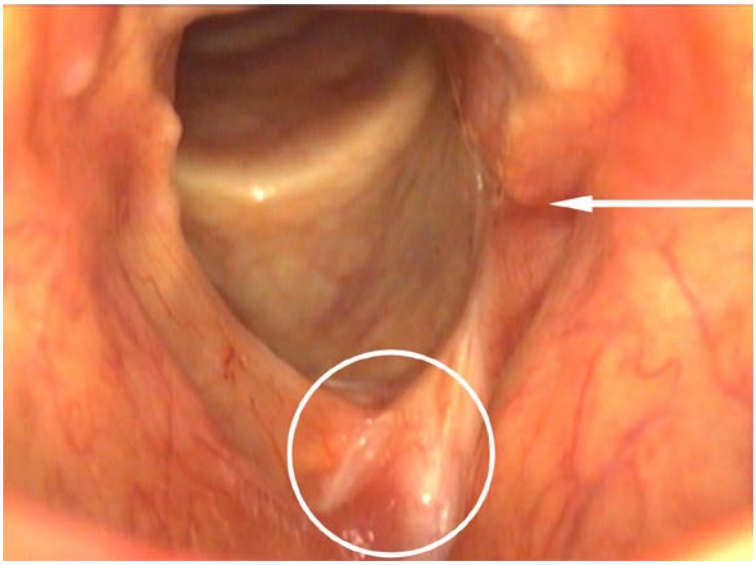

Fig. 1 – Postoperative appearance of the patient's larynx (rigid telelaryngoscopy, 90 degrees): no recurrence was observed, while a small anterior neoglottal web (white circle) and left-sided contact ulcer (arrow) were found as incidental findings.

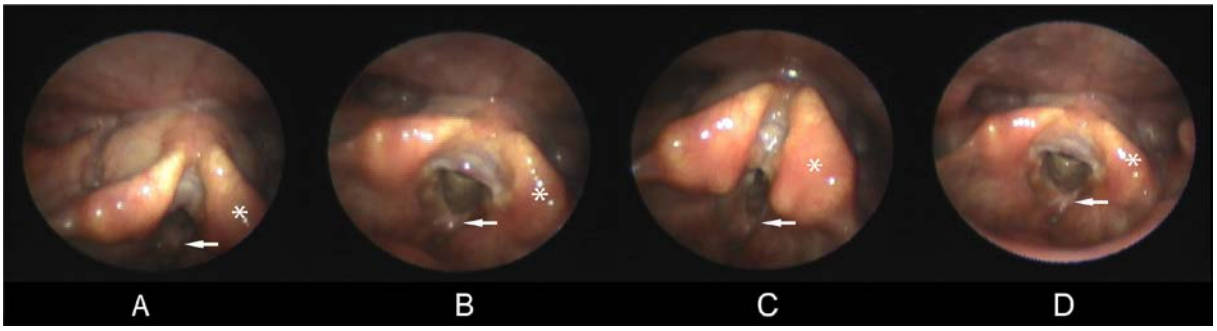

Fig. 2 – Transnasal flexible fiberoptic laryngoscopy during trombone playing showing arytenoids (*) and the neoglottis (arrow). The patient was instructed to play a sustained B-flat in four different octaves: A) Bb3, B) Bb4, C) Bb2, and D) Bb1.

Discussion

The tone of a brass wind instrument is created by the vibrating lips of the performer on the rim of the conical mouthpiece. The vibrations of the lips are generated by exhaled air and amplified by the instrument itself. Tone volume and pitch are regulated by altering the speed and volume of the supporting expiratory airstream and the tension of the perioral muscles. In addition, effective brass instrument playing requires complex coordination of facial and respiratory muscles, as well as movements of the tongue and jaw to shape and direct the airstream. The larynx and vocal folds especially contribute to the modulation of the airflow and musical phrasings, such as staccato, slurring, and vibrato, according to earlier endoscopic research on wind instrumentalists [3–6]. Although the larynx remains structurally intact following RT for early glottic carcinoma, a variety of local side effects, such as transitory or chronic laryngeal edema, xerostomia, or throat dryness, can have a significant impact on tone output in wind instrument players [7]. TLM is currently considered a standard in the surgical treatment of small and midmembranous vocal fold lesions [8]. It has less postoperative morbidity than open procedures, avoids the side effects of RT, and appears to be a feasible treatment choice in our case. Unfortunately, due to insufficient visibility of the endolarynx, this strategy proved to be inapplicable. Vertical partial laryngectomies, including open cordectomy through laryngofissure, imply midline transection of the thyroid cartilage for tumor exposure and removal, therefore disrupting the integrity of the laryngeal skeleton and often resulting in substantial tissue defects. Previously reported local complication rates for organ-preserving surgery for laryngeal carcinoma vary from 8% for open cordectomy to 14% for vertical partial laryngectomy [9]. Regardless of the meticulous surgical technique, subsequent healing of the transected laryngeal framework and epithelization of the resulting defect is often unpredictable. It may cause excessive glottal scarring, webs, stenosis, and granuloma formation, which might hypothetically affect the airflow control required for playing wind instruments. In our case, just a minor granuloma was discovered in the location of the excised vocal process, which did not appear to impact the performance of the player. Since our patient was a member of an orchestra with national significance, the surgeon was additionally challenged to tailor the therapeutic approach to achieve radical surgery while allowing the patient to actively maintain his musical engagement. Cavalot et al. [10] described a case of a professional musician who underwent total laryngectomy for pT3 laryngeal carcinoma and resumed his career following voice prosthesis insertion. Nevertheless, there was no precise description of the patient's professional participation and musical ability before and after therapy. Playing trombone in a major orchestra requires complete technical proficiency with the instrument in terms of range, endurance, and stylistic accuracy, whether performing a 17th-century opera or contemporary ballet music. Our patient was able to respond to these demands as effectively as before surgery. RT and surgery are reported to be equally effective in the treatment of the early stages of laryngeal SCC, and, to date, there is no proof that one modality is superior to the other [11]. Hence, the proper treatment is selected at the discretion of the patient and the physician. The location and size of the tumor, the patient's age, and the presence of comorbidities must be considered while evaluating treatment choices. The final decision should be based on the method with the lowest risk of complications that would interfere with the patient's everyday life and professional life without jeopardizing the oncologic outcome [9, 12]. Our patient assessed that the possible post-radiation xerostomia or throat dryness would have a greater impact on his playing, and he favored surgery.

Conclusion

Due to the scarcity of literature and the possibly harmful effects of therapy on their performance and career, selecting a particular treatment for head and neck cancers in performing musical artists – professional wind players – remains difficult. This article demonstrates the viability of partial open laryngectomies for laryngeal cancer in a professional trombonist without compromising his performance. With more advanced carcinoma, it is unclear how wider partial resections of the larynx would affect the careers of wind instrumentalists; thus, additional study is required in this area.

Acknowledgement

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C A S E R E P O R T

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Satisfying outcome of vagus nerve stimulation applied in the treatment of a patient with drug-resistant epilepsy caused by periventricular nodular heterotopia

Zadovoljavajući ishod primene stimulacije vagusnog nerva u lečenju bolesnika sa epilepsijom rezistentnom na lekove nastalom usled periventrikularne nodularne heterotopije

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Abstract

Introduction. Periventricular nodular heterotopia (PNH) is a developmental malformation of the cerebral cortex characterized by abnormal migration of neurons into the cortical plate and is often associated with drug-resistant focal epilepsy. **Case report.** A 33-year-old female patient suffered from drug-resistant epilepsy, which was predominantly characterized by focal seizures, with occasional seizures that had a focal onset and progressed into bilateral tonic-clonic seizures. Magnetic resonance imaging showed bilateral heterotopic nodules in the occipital horns of the lateral brain ventricles. 18-Fluoro-deoxyglucose positron emission tomography – FDG-PET scan demonstrated a zone of reduced glucose metabolism on the right temporal region. Electroencephalogram suggested focal electrocortical activity on the temporo-parieto-occipital regions, predominantly on the right temporal side. The woman was treated with polytherapy (valproic acid, lamotrigine, levetiracetam, oxcarbazepine, pregabalin, clobazam, and lacosamide), but it did not affect the seizure frequency. Due to the bilateral localization of the heterotopic nodules, surgical treatment was not recommended. After a multidisciplinary assessment, we decided on vagus nerve stimulation (VNS) and achieved satisfying seizure control. **Conclusion.** Patients with PNH require multidisciplinary assessment and treatment, while in this report we have a case of a pa-tient in whom satisfying control of drug-resistant epilepsy was achieved after the implantation of the VNS device.

Key words:
brain; congenital abnormalities; drug-resistant epilepsy; epilepsies, partial; magnetic resonance imaging; positron-emission tomography; vagus nerve stimulation.

Apstrakt

Uvod. Periventrikularna nodularna heterotopija (PNH) predstavlja malformaciju u razvoju kore velikog mozga koja se karakteriše abnormalnom migracijom neurona u kortikalnu ploču i često je povezana sa fokalnom epilepsijom rezistentnom na lekove. **Prikaz bolesnika.** Bolesnica stara 33 godine patila je od farmakorezistentne epilepsije, koju su pretežno karakterisali fokalni napadi, sa povremenim napadima koji su imali fokalni početak i širili se u bilateralne toničko-kloničke napade. Nalazima magnetne rezonance pokazano je da bilateralno postoje heterotopični čvorovi duž okcipitalnih rogova bočnih komora mozga. Nalaz *18-Fluoro-deoxyglucose positron emission tomography* – FDG-PET skenera je pokazao postojanje zone smanjenog metabolizma glukoze u desnom temporalnom regionu. Elektroencefalogram je ukazao na fokalnu elektrokortikalnu aktivnost u temporo-parijeto-okcipitalnim regionima, pretežno temporalno desno. Bolesnica je lečena politerapijom (valproična kiselina, lamotrigin, levetiracetam, okskarbazepin, pregabalin, klobazam, lakozamid) koja nije uticala na učestalost epileptičkih napada. Zbog bilateralne lokalizacije heterotopičnih čvorova, hirurški način lečenja nije bio preporučljiv. Nakon multidisciplinarnog sagledavanja, odlučili smo se za stimulaciju vagusnog nerva (SVN) i postigli zadovoljavajuću kontrolu epileptičkih napada. **Zaključak.** Bolesnici sa PNH zahtevaju multidisciplinarno sagledavanje i lečenje, dok u ovom slučaju imamo primer bolesnice kod koje je postignuta zadovoljavajuća kontrola farmakorezistentne epilepsije nakon implantacije uređaja za SVN.

Ključne reči:
mozak; anomalije; epilepsija, farmakorezistentna; epilepsija, parcijalna; magnetska rezonanca, snimanje; tomografija, pozitron-emisiona; n. vagus, stimulacija.

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Introduction

Neural migration, together with neural proliferation and cortical organization, represents the fundamental processes of neocortex formation, whose end result is the establishment of a functional cerebral cortex. Inadequate migration and organization of the laminarly structured cortex causes congenital malformations of cortical development [1]. Periventricular nodular heterotopia (PNH) is a rare malformation of cortical development characterized by abnormal migration of neurons to the cortical plate. It is characterized by nodular masses, which protrude into the ventricular lumen. The most common localization is along the occipital horns of the lateral ventricles. PNH is frequently characterized by drug-resistant epilepsy (DRE) and different forms of mental retardation. PNH can be presented as bilateral and symmetrical, bilateral single-noduled, bilateral and asymmetrical, and unilateral with extension to neocortex and unilateral. In more than 75% of cases, the nodules are localized bilaterally [2].

Magnetic resonance imaging (MRI) and 18-fluorodeoxyglucose positron emission tomography - FDG-PET scans play an important role in the recognition and diagnosis of PNH [3, 4]. Vagus nerve stimulation (VNS) should be considered for the treatment of severe DRE, especially if the patients are not candidates for surgical intervention [5]. Hereinafter, we report a case of a patient with satisfying seizure control after the VNS device implantation.

Case report

A 33-year-old woman with a history of DRE was admitted to the hospital for clinical and therapeutic assessment. The patient was born from a normal, full-term pregnancy *via* vaginal delivery and without any perinatal complications. The mild mental retardation was diagnosed in childhood. There was no family history of seizures. The seizures occurred when she was 14 years old, with the frequency of four to five times a day, lasting less than 2 min. She had an epilepsy characterized by focal seizures, occasionally bilateral tonic-clonic seizures. The mother usually noticed that the patient had speech difficulties, could not pronounce words clearly, could not establish contact with her (did not answer calls, and acted as "lost"). This would usually be followed by convulsions in the left arm and leg. Occasionally, the patient reported the feeling of epigastric heat that would radiate cranially. At a later stage of the disease, the seizures took on a new pattern. Moreover, the patient became aggressive during the seizures, even running off on a couple of occasions. Polycystic ovary syndrome (PCOS), diabetes mellitus (DM) type 1, hypothyroidism, and thrombophilia were diagnosed in our patient. Her comorbidities have significantly limited our therapeutic options. Valproic acid (1,000 mg/day) partially controlled the seizures until she was 16 years old when, due to the irregularity of the menstrual cycle, the PCOS was diagnosed. Henceforth, she did not take hormone therapy for PCOS regularly. For those reasons, valproic acid was replaced by lamotrigine (100 mg/day). In addition, the convulsions continued to be frequent, so levetiracetam (1,000 mg/day) was added to the therapy, but without any improvement in the patient's condition. Furthermore, lamotrigine had to be excluded from the therapy due to interaction with hormone therapy. On the other hand, carbamazepine has not been added to therapy due to thrombophilia and the use of warfarin. The seizures became more frequent and complex. Several scars from burns sustained after the seizures were registered on her left hand, and as a result of one convulsion, the patient also suffered serial rib fractures. Her illness caused a severe functional disability.

During the initial hospital examination, the results of the clinical examination were normal. Electroencephalogram (EEG) findings indicated focal electrocortical activity predominantly on the right temporal region (Figure 1). MRI demonstrated bilateral nodular and confluent changes in the occipital horns of the lateral ventricles, which corresponded to ectopic gray matter and indicated congenital cortical malformation, micronodular periventricular gray matter heterotopia (Figure 2). Such a finding was confirmed by the FDG-PET scan (Figure 3), where a zone of reduced glucose metabolism was registered in the right temporal region (polar, mesial, and lateral).

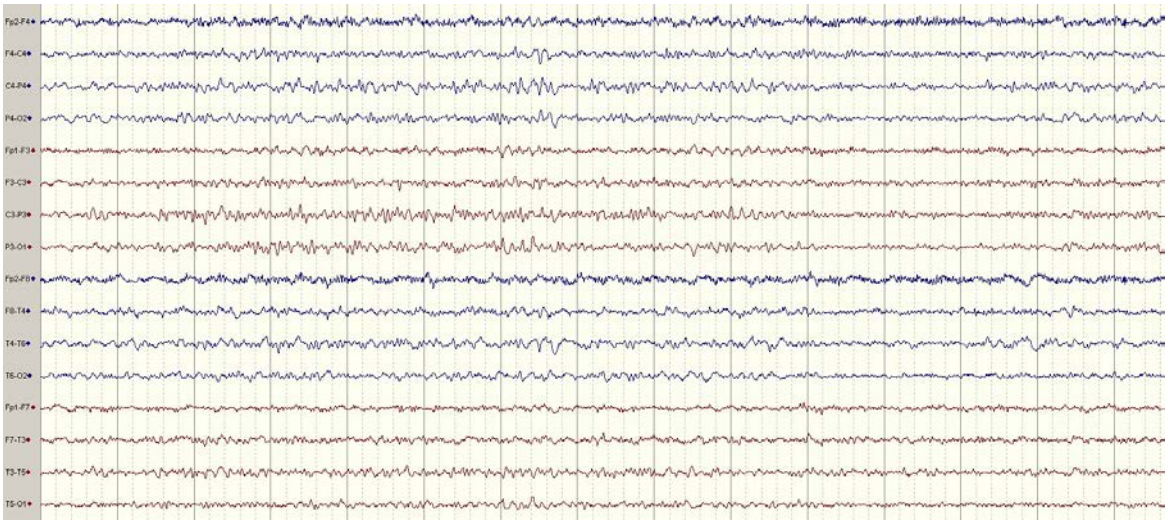

Fig. 1 – Electroencephalogram showing focal electrocortical activity on the temporo-parieto-occipital regions, predominantly on the temporal right.

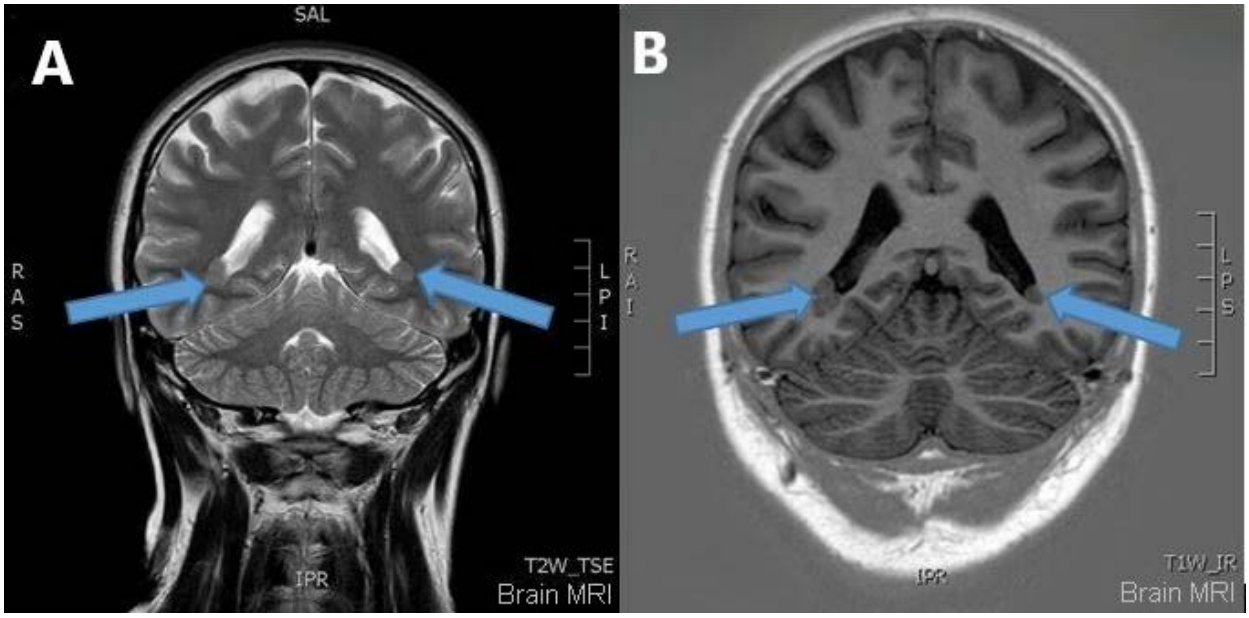

Fig. 2 – Magnetic resonance imaging: A) T2 weighted turbo-spin-echo (T2W TSE); B) T1 weighted inversion-recovery (T1W IR); para-coronal tomograms (A, B) orthogonally oriented to the hippocampus showing bilateral heterotopic nodules of grey matter in the occipital horns of the lateral ventricles (arrows). RAS – right-anterior-superior; LPI – left-posterior-inferior; SAL – superior-anterior-lateral; IPR – inferior-posterior-right; RAI – right-anterior-inferior; LPS – left-posterior-superior.

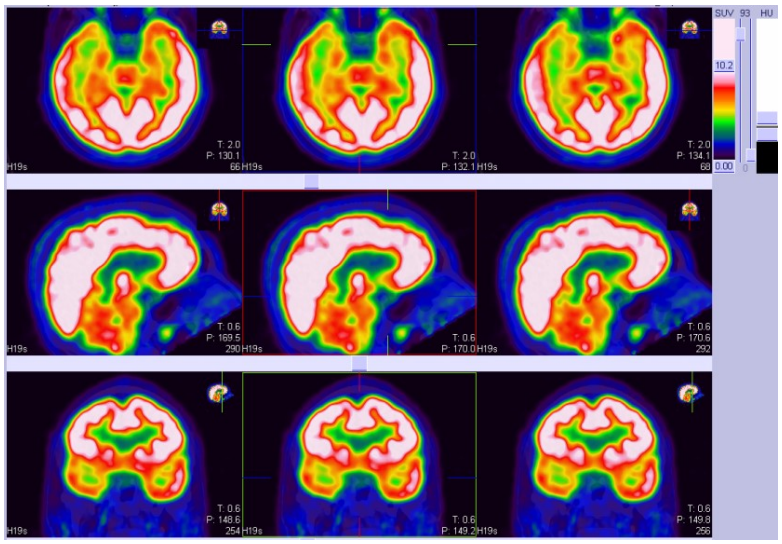

Fig. 3 – 18-Fluoro-deoxyglucose positron emission tomography – FDG-PET, shows a zone of reduced glucose metabolism in the temporal region. The scan indicates high (red) to low (blue) tracer uptake or binding. Images in the upper row represent axial, in the middle row sagittal, and in the lower row coronal plane sections.

Neuropsychological testing (NPT) was also performed. The analysis of the results of the NPT recorded a lower primary intellectual level and educational limitations, as well as a subdepressive effect, with the registration of a drop in visual memory, dysexecutive syndrome on visual material, as well as reduced processing speed. NPT indicated dysfunction of the non-dominant hemisphere.

The seizures continued to occur frequently (several seizures a day), and the patient was hospitalized on several occasions. At her follow-up examinations, the dose of medication was increased (levetiracetam 4,000 mg/day), and other antiepileptic drugs (oxcarbazepine 2,400 mg/day, pregabalin 600 mg/day, clobazam 30 mg/day, lacosamide 300 mg/day) were introduced into the therapy. Even with such polytherapy, the seizures were still frequent. Surgical treatment was controversial due to the bilateral localization of heterotopic nodules around the occipital horns of the lateral ventricles, as well as due to the high risk of visual impairment. Therefore,

the VNS device was implanted (Figure 4). The follow-up examinations were performed three and six months later, as well as one year after the intervention, and there was a significant reduction in the frequency of seizures during that period. A notable decline was observed in the occurrence rate of epileptic seizures, approximately two to three times a week. In the case of our patient, VNS proved to be an appropriate therapeutic modality, given that we achieved satisfying seizure control.

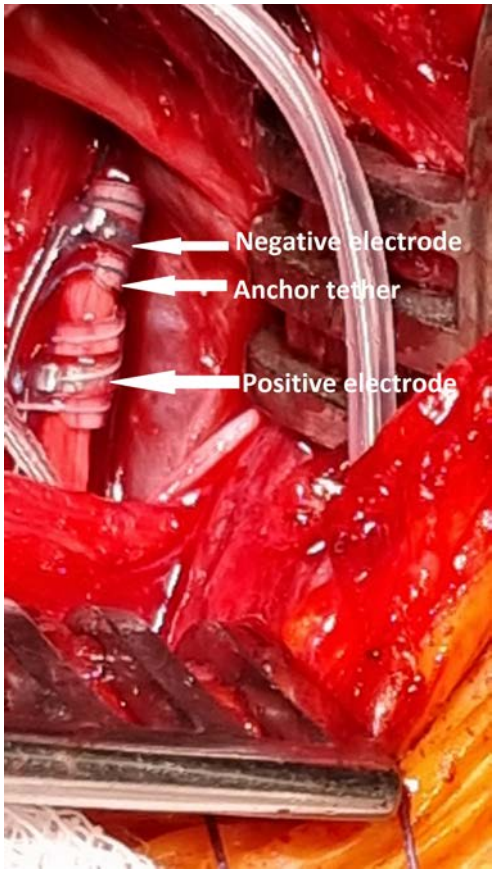

Fig. 4 – Surgical implantation of the vagus nerve stimulation device.

Discussion

We present a patient with DRE due to PNH with numerous severe comorbidities such as DM type 1, PCOS, thrombophilia, and hypothyroidism in whom successful control of epileptic seizures was achieved after the implantation of the VNS device.

The classic form of PNH is often manifested as epilepsy. In addition to epilepsy, some patients may have dysmorphic facial features and intellectual disabilities, which are predominantly of a milder degree. The severity of epileptic seizures and especially mental retardation depends on the degree of cortical abnormality [6]. Raymond et al. [7] reported 13 patients with periventricular heterotopia and epilepsy. The time of onset of the seizures was between 18 months and 20 years of age. Eight patients had bilateral PNH, while five had the unilateral form. Several patients from the bilateral group were similar to ours (from a clinical point of view, EEG and neuroimaging). Similarly, Dubeau et al. [8] reported a study of 33 patients with PNH. Fourteen patients had bilateral lesions. Some of them had normal intellectual and motor functions (64.3%), but some had mild mental retardation (35.7%). Recurrent seizures occurred in 82% of cases. In most of the patients, those were focal seizures with aura. Several of those patients were similar to ours if we consider radiological and clinical expression of malformation.

D'Orsi et al. [9] compared the outcomes of the two groups of patients with malformations of cortical development. Their report included 120 patients, among which 16 of them had PNH. One group included patients with simple PNH without other cortical or cerebral malformation (eight patients). Another group included patients with other cortical or cerebral malformations (eight patients), such as subcortical heterotopia, polymicrogyria, focal dysplasia, schizencephaly, cortical infolding, agenesis of the *corpus callosum*, *mega cisterna magna*, and cerebellar atrophy (PNH plus). The group without associated malformations was usually characterized by normal intelligence and seizures, usually focal, that began during the second decade of life. Seizures were usually rare. The EEG showed focal abnormalities. In the group of patients with associated malformations, seizures were very frequent in most cases, while these patients had mental retardation. The EEG in this group showed focal and bisynchronous abnormalities. This report indicated that the presence of other types of cortical or cerebral malformations, in addition to PNH, determines a poor prognosis. In our case, we had patients without other proven malformations, but seizures were very frequent and associated with mild mental retardation. Gray matter heterotopias are difficult to differentiate from hamartomas of tuberous sclerosis, and MRI is usually required to clarify the diagnosis [10]. On the other hand, the FDG-PET scan illustrates a disorder of glucose metabolism in heterotopic gray matter. This diagnostic method precisely identifies epileptogenic foci, which is crucial for surgical planning and good postoperative seizure control [11]. A pilot study on 19 patients with PNH showed that heterotopic nodules were hypometabolic in 33.33% of patients and that 83.33% of patients with PNH had concordant FDG-PET/MRI findings with the clinical epileptic zone [11]. In our case, clinical and EEG characteristics of the seizures indicated involvement of the temporal lobe. Furthermore, MRI imaging and FDG-PET scans helped us significantly in making the final diagnosis. The complexity of our case was also reflected in the therapeutic limitation. Namely, our patient had a number of therapeutic limitations due to comorbidities. PCOS, thrombophilia, as well as the use of hormone therapy significantly limited our therapeutic options. For these reasons, we had additional difficulties in controlling the seizures. Regarding the data on long-term effects of VNS, the results of a Norwegian population-based study indicated a gradual increase in the beneficial effects of VNS over time, expressed as a cumulative probability of more than 50% reduction in seizure frequency, which was found in almost

60% of patients. Particularly significant results were achieved in a subgroup of patients without intellectual disabilities, with a pronounced probability of achieving a ≥ 75% reduction in seizure frequency. Within specific epileptic conditions, the most pronounced beneficial effects were observed in patients with poststroke epilepsy (75.0%) and post-traumatic epilepsy (70.6%) [12]. Ghaemi et al. [13] reported a study with 144 patients with some of the following features: multifocal interictal epileptiform discharge, unilateral interictal epileptiform discharge, cortical dysgenesis, and psychomotor seizure. Results of their study showed that ten patients remained seizure-free for more than one year after VNS device implantation (6.9%). Seizure frequency improved in 61.8% of patients, but no changes were observed in 31.3% of patients. Moreover, some studies indicated that VNS is a suitable treatment for DRE and can reduce polypharmacy during pregnancy [14].

The opinion of the Council for Functional Neurosurgery was that the operative treatment in our patient was not an appropriate treatment modality due to the bilateral localization of heterotopic nodules around the occipital horns of the lateral ventricles, as well as due to the high risk of visual impairment. Some authors believe surgery should be performed in cases where heterotopic nodules are located outside the eloquent areas and unilaterally [15]. On the other hand, some authors suggest the importance of stereotaxic ablative procedures in the treatment of PNH; however, we did not opt for them due to the high degree of potential occurrence of neurological deficits after these procedures, as well as insufficient data from the scientific literature [16].

Conclusion

In conclusion, significant advances in neuroimaging techniques have improved our knowledge of malformations in cortical development. Evaluation of clinical features and long-term follow-up of these patients is essential but is often neglected. Our case presents PNH as a common cause of DRE. Furthermore, this case emphasizes the difficulties of treating patients with PNH, as well as the role of MRI imaging in recognizing this anomaly. VNS plays an important role in achieving successful control of epileptic seizures. Early recognition of heterotopia is essential for planning the proper treatment.

Conflict of interest

The authors report no conflict of interest and no sources of support that require acknowledgment.

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The oldest apothecaries of the Serbian Armed Forces

Najstariji apotekari srpske vojske

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Key words:
history, 19th century; history of medicine; history of pharmacy; military medicine; pharmacists; military personnel; serbia.

Ključne reči:
istorija, xix vek; istorija medicine; istorija farmacije; medicina, vojna; farmaceuti; kadar, vojni; srbija.

Introduction

After the Sultan's second Hatt-i Sharif in 1830, the emerging young Serbian state gained the right to establish and develop health facilities. Soon after, in the following years, the first pharmacy facilities were founded. In Belgrade in 1830, Matija Ivanović from Zemun opened a private pharmacy on the first floor of a building that exists to this day – tavern "?" ("Question Mark"). In Kragujevac in 1836, Pavle Ilić from Veliki Bečkerek (Zrenjanin today) opened a state pharmacy "For the Prince and the Guard", which also supplied medicine to a small guard's hospital. The pharmacy was moved to Belgrade in 1839 for a short time (for one year) and then permanently in 1841, under a changed name, "Government Pharmacy". It continued working as such until June 10, 1859, supplying medicines to the Military Hospital. After that, it was sold to a natural person (MPharm Teodor Sekulić), and Pavle Ilić was appointed state chemist and manager of the State Chemical Laboratory. Anton Delini, an empiricist from Zemun, had his pharmacy shop for the medicine trade and sold medicines to the people of Belgrade. In 1844, Delini tried to open a pharmacy shop for the medicine trade in Dorćol, but he only succeeded in 1860 when his son Andrija returned from Vienna with a master's degree in pharmacy. Before all of them, Prince Jevrem Obrenović opened a pharmacy in Šabac in 1826, where MPharm Jovan Mioković worked. Until these pharmacies were opened, the few doctors who were active in Serbia used their own private medical kits supplied from neighboring Austria, while various herbs and mixed chemicals were sold to the people by merchants, usually Greeks, Cincars, and Jews. In addition, the people were treated by various herbalists, healers, and soothsayers.

Uvod

Mlada srpska država u nastajanju, posle drugog sultanovog hatišerifa iz 1830. godine, stekla je pravo da osniva i razvija zdravstvene ustanove. Ubrzo, tokom sledećih godina, u njoj se osnivaju prve apotekarske ustanove. U Beogradu 1830. godine, Matija Ivanović iz Zemuna otvara privatnu apoteku na prvom spratu zgrade koja i danas postoji (kafana „?" – „Znak pitanja"). U Kragujevcu 1836. godine, Pavle Ilić iz Velikog Bečkereka (današnji Zrenjanin) otvara državnu apoteku („Knjaževska i gvardejska") koja je snabdevala lekovima i malu gvardejsku bolnicu. Ova apoteka je 1839. godine prvobitno na kratko (godinu dana), a zatim 1841. godine za stalno prešla u Beograd pod izmenjenim imenom „Praviteljstvujušća apoteka". Kao takva, radila je do 10. juna 1859. godine, snabdevajući lekovima Vojnu bolnicu. Nakon toga, prodata je privatnom licu (mr farm. Teodor Sekulić), a Pavle Ilić postavljen je za „državnog kemika" i upravnika Državne hemijske laboratorije. Anton Delini, empirik iz Zemuna, imao je svoju trgovinu lekovima i prodavao lekove Beograđanima. Delini je pokušavao da otvori trgovinu lekovima na Dorćolu 1844. godine, ali mu je to uspelo tek 1860. godine, kada mu se sin Andrija vratio iz Beča sa diplomom magistra farmacije. Pre svih njih, knez Jevrem Obrenović je u Šapcu 1826. godine otvorio apoteku u kojoj je radio magistar Jovan Mioković. Do otvaranja tih apoteka, ono malo lekara koje je bilo aktivno u Srbiji koristilo je svoje privatne priručne apoteke kojima su se snabdevali iz susedne Austrije, dok su narodu razne trave i smućkane hemikalije prodavali trgovci, obično Grci, Cincari i Jevreji. Pored njih, narod su lečili raznorazni travari, hećimi i bajalice.

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As for the Army, that is, at the beginning of the so-called Guard, after the imposition of the "Turkish" Constitution in December 1838, the first National Army was formed. It was an embryo that would only later, during the time of Prince Mihailo, gradually develop and become the real People's Army with a small core of permanent members. Its first military doctors, doctors of medicine, and masters of surgery will manage in the same way as their civilian counterparts – by using, among other things, the help of these rare pharmaceutical institutions.

In 1839, a constitutional military organization was established with 17 infantry companies and one half-battery of artillery, in which, by the Prince's Decree of July 18/31 of the same year, Dr. Emmerich Lindenmayer from Banat Swabia was appointed as a member of the Main Military Staff and Staff Doctor, i.e., the first head of military health, who served in Kragujevac together with the Prince. Soon after, the patron of surgery, Moritz Fiedler, a long-time Austrian military doctor, was appointed as his apprentice for the Belgrade garrison and hospital. Shortly after Fiedler's death, the master of surgery and pharmacy, Florian Birg, a Hungarian, was appointed as the "first staff surgeon of the army" on October 17, 1841. Until then, Birg was a doctor of the Belgrade city service of public health, and since then, he has been the doctor of the Belgrade garrison and its hospital.

According to his schooling and 16 years of practice before coming to Serbia, Birg would have been the first trained apothecary in the Serbian military service, although it remains questionable whether he effectively performed that service together with the medical (military medicine), which, according to the available data, was prevailing. If, due to this fact, he is not taken into account, the first effective apothecary of the Serbian Army would be MPharm Josif Katrain, and the first person who raised military pharmaceutics to higher levels, introducing more serious and high-quality laboratory-diagnostic activity into it, would undoubtedly be master of pharmacy and chemistry, Jaroslav Helih.

As the limit for the title "oldest military apothecaries of the Serbian Armed Forces", we took the year 1876, the beginning of the First Serbian-Ottoman War. During the war itself, in addition to a large number of medical personnel, a considerable number of pharmacists also rushed to Serbia, some of whom remained in Serbia even after the war. In this way, we thought the eight people we are portraying would get their rightful place in the history of Serbian military medical services. In addition, we have to mention that it is surprising that the Serbian military pharmacy as a whole has not received more research attention. So far, the most famous book on this topic was written in 1977 by Professor Dragan Stupar, "Military Pharmacy of Serbia in the 19th Century", which stems from his doctoral dissertation defended at the Faculty of Pharmacy in Belgrade in 1975 [1–4].

Što se tiče vojske, tj. u početku gvardije, nakon nametnutog „turskog" Ustava iz decembra 1838. godine došlo je do formiranja prve narodne vojske tj. nekog embriona koji će tek kasnije postepenim razvojem, u vremenima Knjaza Mihaila postati prava narodna vojska sa malim jezgrom stajaće vojske. Njeni prvi vojni lekari, doktori medicine i magistri hirurgije, snalaziće se na isti način kao i njihove kolege iz građanstva – koristeći, između ostalog, i pomoć ovih retkih apotekarskih ustanova.

Godine 1839, ustanovljena je Ustavna vojna organizacija sa 17 pešadijskih četa i jednom polubaterijom artiljerije, u kojoj je Knjaževskim ukazom od 18/31 jula iste godine banatski Švaba dr Emerih Lindenmajer postavljen za člana Glavnog vojnog Štaba i štab-doktora, t.j. prvog šefa Vojnog saniteta koji je stolovao u Kragujevcu pored Knjaza. Uskoro je za njegovog pomoćnika za beogradski garnizon i bolnicu bio imenovan patron hirurgije Moric Fidler, dotle dugogodišnji austrijski vojni ranar. Ubrzo posle Fidlerove smrti, za „prvog štab hirurga vojinstva" primljen je 17. oktobra 1841. godine magistar hirurgije i farmacije Florijan Birg, Mađar, dotle lekar beogradskog fizikata, a od tada beogradskog garnizona i njegove bolnice.

Po školskoj spremi i 16-godišnjoj praksi pre dolaska u Srbiju, Birg bi bio prvi školovani apotekar u srpskoj vojnoj službi, mada ostaje upitno da li je on tu službu efektivno i vršio zajedno sa medicinskom (vojnolekarskom), koja je, prema raspoloživim podacima, bila preovlađujuća. Ukoliko se zbog te činjenice on ne uzme u obzir, prvi efektivni apotekar srpske vojske bio bi mr farm. Josif Katrain, a prva ličnost koja je vojnu famaciju uzdigla na više vrednosti, unoseći u nju i ozbiljniju i kvalitetniju laboratorijsko-dijagnostičku delatnost, bio bi, nesumnjivo, mr farm. i hemije Jaroslav Helih.

Kao granicu oznake „najstariji vojni apotekari srpske vojske" uzeli smo 1876. godinu do početka Prvog srpsko-turskog rata. U toku samog rata, u Srbiju je, pored velikog broja medicinskih ličnosti, pohrlio i znatan broj farmaceuta, od kojih su se neki zadržali u Srbiji i posle rata. Smatrali smo da bi na ovaj način osmorica koje prikazujemo dobili svoje pravo mesto koje im u istoriji srpskog vojnog saniteta pripada. Uz to, mora se napomenuti da je začuđujuće da srpsko vojno apotekarstvo, u celini gledano, nije dobilo više istraživačke pažnje. Do sada, najpoznatija je knjiga prof. Dragana Stupara „Vojna farmacija Srbije u XIX veku" iz 1977. godine, koja proističe iz njegove doktorske disertacije, odbranjene na Farmaceutskom fakultetu u Beogradu 1975. godine [1–4].

Florian Birg – "Cvetko" (Pest?/Szeged! 1801 – Belgrade, October 13, 1865)

It is not clear what Florian Birg's place of birth was – his conduit list from 1844 speaks of Pest, where to the question: "Where are you from?" he answered: "From Pest", and the information from the Register of Deaths of the Roman Catholic Apostolic Church in Belgrade indicates that Szeged was his place of birth. Anyhow, he completed elementary school and a six-year gymnasium in his hometown and "philosophy" in Pest (seventh and eighth grade of the higher gymnasium), where he also obtained his master's degree in medicine, surgery, and pharmacy. Following these events, for 16 years, he was a private apothecary in Pest, and then, in 1837, he came to Belgrade to serve in the Turkish garrison as "the second doctor and apothecary in the hospital pharmacy". Two years later, on February 14, 1839, he transferred to the Serbian service in the position of the Belgrade primary supervising physician. Later, as mentioned above, after the death of the first staff surgeon, Moritz Fiedler, Florian Birg took his place on October 17, 1841.

Birg remained in the position of the first staff surgeon until 1859. In all likelihood, he was a kind of medical "two-headed" and perhaps "multi-headed" mythical creature, serving as both a physician's apprentice and apothecary and working in Kragujevac and Belgrade. For his first service as a physician's apprentice, there are several written proofs, such as the one of Dr. Vladan Đorđević in his first book, "History of Serbian Military Health", from which it can be seen that Birg treated sick soldiers and submitted reports about it. For his second service as an apothecary, we cannot find any confirmation, although we consider it logical and true, bearing in mind his pharmacy diploma and 16 years of experience, as well as the weak staffing opportunities that prevailed in Serbia at that time. Perhaps this is supported by the fact that on November 15, 1847, he resigned from the military service and at the same time begged for any other service "because he had too little reward and too much work" without specifying anything further. Nevertheless, his service in Serbia was primarily a medical one, as evidenced by the fact that when his request was finally granted in 1859, he was appointed again as the primary supervising physician of the Belgrade district, a position he held until his death. He was also the acting manager of the Psychiatric Hospital "*Dom za s' uma sišavših*" from its establishment on August 3, 1861, as can be seen from his application for the payment of fees (in the Review of Government Affairs from 1867, Dr. Mladen Janković appears in that position). Upon his return from exile, Prince Miloš promoted him to the rank of staff captain, which was announced in "*Novine serbske*" on April 18, 1859. Finally, as early as 1844, together with Dr. Lindenmayer, Birg founded the "Museum of Rarities" in one wing of the old Palilula barracks, which can be considered the first natural history and medicine museum in Serbia.

According to his official assessment entered in the military conduit list for 1844, he was "naturally gifted, good, sociable, polite, rather strict, quite agile, punctual, diligent, with a small body, and of good health".

Florijan Birg – „Cvetko" Pešta?/Segedin! 1801 – Beograd, 13. oktobar 1865)

Nije jasno koje je mesto rođenja Florijan Birga – za Peštu govori njegova konduit lista iz 1844. godine, gde na pitanje „Odakle je?", on odgovara „Iz Pešte", a da se rodio u Segedinu govori podatak iz Matične knjige umrlih apostolske rimokatoličke misije u Beogradu, u kojoj je kao mesto rođenja upisan Segedin. U svakom slučaju, u rodnom mestu je završio osnovnu i šestorazrednu gimnaziju, a u Pešti „filosofiju" (sedmi i osmi razred više gimnazije), kao i magistarske, mediko-hirurške i apotekarske studije. Potom je u Pešti bio 16 godina privatni apotekar, da bi 1837. godine došao u službu turskog garnizona u Beogradu kao „drugi lekar i apotekar u bolničkoj apoteci". Nakon dve godine, 14. februara 1839. godine, prešao je u srpsku službu na mesto beogradskog fizikusa. Kasnije, kao što je rečeno, po smrti prvog štab-hirurga Morica Fidlera, na njegovo mesto prešao je Florijan Birg, 17. oktobra 1841. godine.

Na položaju prvog štab-hirurga ostao je do 1859. godine. Po svoj prilici, Birg je bio neka vrsta medicinskog „dvoglavog", a možda i „višeglavog" stvorenja, vršeći službu i lekarskog pomoćnika i apotekara, radeći i u Kragujevcu i Beogradu. Za prvo imamo na više mesta pisanje dr Vladana Đorđevića u njegovoj prvoj knjizi „Istorija srpskog vojnog saniteta", iz kojeg se vidi da je lečio obolele vojnike i o tome podnosio izveštaje. Za drugo ne nalazimo potvrde, iako smatramo logičnim da je tako i bilo, imajući u vidu njegovu apotekarsku diplomu i 16-godišnje iskustvo, kao i slabe kadrovske mogućnosti, koje su tada bile priustne u Srbiji. Možda u prilog tome govori činjenica da je 15. novembra 1847. godine podneo ostavku na vojnu službu i pri tome molio za ma kakvu drugu službu „jer ima isuviše malu nagradu, a suviše veliki posao", ne navodeći pri tome ništa bliže. Ipak, njegova služba u Srbiji bila je prevashodno lekarska, o čemu govori i činjenica da je, kada je najzad udovoljeno njegovoj molbi, 1859. godine, ponovo postavljen za fizikusa beogradskog okruga, mesto na kome je ostao sve do smrti. Bio je i vršilac dužnosti upravnika psihijatrijske ustanove „Dom za s'uma sišavših" od osnivanja 3. avgusta 1861. godine, što se vidi iz njegove molbe za isplatu honorara (već 1867. godine se na tom mestu u državnom šematizmu pojavljuje dr Mladen Janković). Knjaz Miloš ga je po povratku iz egzila unapredio u čin štab-kapetana, o čemu je u „Srbskim novinama" izašla anonsa 18. aprila 1859. godine. Najzad, još 1844. godine, Birg je zajedno sa dr Lindenmajerom osnovao u jednom krilu stare palilulske kasarne „Muzeum retkosti", što se može smatrati prvim prirodnjačko-medicinskim muzejom u Srbiji.

Prema njegovoj službenoj oceni upisanoj u vojnoj konduit listi za 1844. godinu, bio je „prirodni darovanija dobri, druževan, učtiv, dosta strog, dosta okretan, točan, prilježan, tela po malenog i zdravlja dobrog".

He died in Belgrade on October 13, 1865. He was married twice. With his first wife, Pulherija, who died while he was still in Turkish service (1838), he had a daughter, Persida. With his second wife, Ana, he had a son, Ljubomir, who changed his religion to Orthodox, and after changing his surname to Birgović, Ljubomir became a professor of natural sciences at Serbian gymnasiums in Užice, Svilajnac, and Čačak, where he also held the position of the high school director* [5–10].

Đorđe Đorđević (Fourka/Epirus, February 15, 1814 – Belgrade, January 7, 1896)

Đorđe Đorđević was of similar fate as Florian Birg in terms of bivalent service. Although he was a trained empiricist, physician, and apothecary's apprentice, he was far more famous as the father of one of the greatest Serbian military and civilian doctors, mayor of Belgrade, law writer, ambassador, minister, and prime minister, Dr. Hipokrat (Vladan) Đorđević.

In his hometown, he completed "three grades of Greek school", then he apprenticed in pharmacies in the Turkish Empire, learning the trade. In Constantinople, he worked for seven years, first as a civilian, then in a military pharmacy. Following his destiny, he finally came to Belgrade, where he felt at home, married the beautiful Maria of the Cincar family Leko, and built a house on Gospodska Street (now Brankova). He worked in the Turkish service in a pharmacy located in the Belgrade fortress. After that, on July 10, 1845, he joined the Serbian Army as a garrison apothecary and continued serving in the Belgrade and Kragujevac garrisons until May 1854, when he resigned. In one source, he states: "My first appointment in 1849 was changed to the position of a chief servant, and for that, my future was placed in the greatest uncertainty". In another source he states: "Due to the process that was conducted by the Ministry of the Interior Affairs against the head of health during my absence, I had my own pharmacy in Sarajevo for two years and five months". The pharmacy, which he and his brother Nikola named "The Lion's Pharmacy", is considered the first modern pharmacy in Bosnia and Herzegovina. In 1857, he entered the military service again. From then until his retirement, he did not leave it ever again. During the wars, he served in the reserve Military Hospital in Ćuprija (1876), Smederevo (1877/78), and in the Belgrade City Command (1885), although he was already an old man and was retired at the age of 71. Officially, he was a medical second lieutenant from September 21, 1859, medical apprentice first

Umro je u Beogradu, 13. oktobra 1865. godine. Bio je dva puta ženjen. Sa prvom suprugom Pulherijom, umrlom još dok je bio u turskoj službi (1838), imao je ćerku Persidu. Sa drugom suprugom, Anom, imao je sina Ljubomira, koji se pokrstio i kao Birgović kasnije bio profesor prirodnih nauka po srpskim gimnazijama u Užicu, Svilajncu i Čačku, gde je bio i direktor† [5–10].

Đorđe Đorđević (Furka/Epir, 15. februar 1814 – Beograd, 7. januar 1896)

Đorđe Đorđević bio je slične sudbine kao Florijan Birg po bivalentnoj službi. Iako priučeni empiričar, lekarski i apotekarski pomoćnik, Đorđe Đorđević je daleko više poznat kao otac jednog od najvećih srpskih lekara, vojnih i građanskih, gradonačelnika Beograda, zakonopisca, ambasadora, ministra i predsednika vlade, dr Hipokrata (Vladana) Đorđevića.

U rodnom mestu je završio „tri razreda grčke škole", zatim je šegrtovao po apotekama u turskom carstvu, učeći zanat. U Carigradu je radio sedam godina, prvo u građanskoj, a potom u vojnoj apoteci, da bi najzad došao, sledeći sudbinu, u Beograd, gde se brzo odomaćio i oženio lepom Macom iz cincarske porodice Leko i okućio, ozidavši kuću u ondašnjoj Gospodskoj ulici (sada Brankova). Radio je u turskoj službi u beogradskoj tvrđavi u apoteci, odakle je u srpsku vojsku stupio 10. jula 1845. godine kao apotekar garnizona i nastavio da služi u beogradskom i kragujevačkom garnizonu do maja 1854. godine, kada je dao ostavku. U jednom izvoru navodi „što prvašnje naimenovanje moje od 1849. godine u ono glavnog poslužetelja preobraćeno i črez toga budućnost moja u najveću neizvesnost stavljena je". Na drugom mestu kaže „zbog gonjenja načalnika saniteta pri popečiiteljstvu vnutreni dela, za vreme odsustvovanja bio sam sobstveni apotekar u Sarajevu dve godine i pet meseci". Apoteka, koju su on i njegov brat Nikola krstili „Apoteka kod lava", smatra se prvom savremenom apotekom u Bosni i Hercegovini. U vojnu službu je ponovo stupio 1857. godine. Od tada, do penzije, nije iz nje izlazio. Za vreme ratova služio je u rezervnoj Vojnoj bolnici u Ćupriji (1876), Smederevu (1877/78) i u Komandi grada Beograda (1885), iako već starac i penzioner, u 71-oj godini. Zvanično, bio je sanitetski potporučnik od 21. septembra 1859. godine, lekarski pomoćnik prve klase od 1. januara 1866. godine, a u čin poručnika preveden 1. marta 1876. godine.

*The given notes exist in the Serbian Biographical Dictionary, published by Matica srpska, Novi Sad, 2004, 550–551. Birg's biography was written by Dr. Teodor Kovač and MPhil Milica Bujas, according to whom he became a master of the science of childbirth and veterinary medicine during his studies. For the latter, they refer to the work of the famous historian of medicine and veterinary medicine, Dr. Dragoljub Divljanović, "Veterinary personnel in Serbia 1800–1918", Belgrade, 1974, p. 23. By the way, according to them, he was born in Pest in 1805, which is an incorrect date, considering his conduit list from 1844, according to which he was 43 years old at that time.

† Navedeni podaci postoje u Srpskom biografskom rečniku, Matica srpska, Novi Sad, 2004, 550–551, Birgova biografija koju su pisali dr Teodor Kovač i mr fil. Milica Bujas, po kojoj je on tokom studija postao i magistar babičluka i veterine. Za ovo poslednje se pozivaju na rad poznatog istoričara medicine i veterine veterinara dr Dragoljuba Divljanovića „Veterinarski kadrovi u Srbiji 1800–1918", Beograd, 1974, 23. Usput, prema njima, on se rodio u Pešti 1805. godine, što je netačan datum, imajući u vidu njegovu konduit-listu iz 1844. godine po kojoj je tada imao 43 godine.

class from January 1, 1866, and was promoted to the rank of lieutenant on March 1, 1876.

Agile and resourceful, educated for the bazaar of the time (he spoke Serbian, Greek, Vlach, Cincar, Turkish, and "honorably German"), and he did well in the army, as can be seen from the official evaluation given to him by the assistant chief of the Serbian health department, Dr. Filip Taisić: "He is (naturally) gifted, very bright, and understanding. In his duties, he is capable, very useful, and reliable... he is an empiricist, skilled in the dispatch of medicines, and does the job of a medical apprentice very well. In his service, he is very zealous, accurate, and attentive." This can be seen from the awards he received: the 5th Order of the Cross of Takovo, Silver Medal for Zealous Service, and Red Cross Medal of Merit.

He died in Belgrade, having lived to see the glory of his only son, whom he was proud of [11–15].

Josif Katrain (Vinkovci, February 11, 1823 – Belgrade, July 16, 1872)

Josif Katrain was born in a large family (three male and two female children) to father Ivan, a carpenter, and mother Katarina, born Presnek. After finishing elementary school and a six-grade gymnasium in his hometown, he completed two years of pharmacy studies, probably in Pest or Prague. His name was first mentioned in Serbia in 1855 in Review of Government Affairs as the first apprentice of the apothecary Pavle Ilić (the second was Radosav Šimić) in the "Government Pharmacy", a pharmacy under the Administration of the city of Belgrade, until June 10, 1859, when it was sold to MPharm Teodor Sekulić and Pavle Ivić was moved to the position of state chemist fifth class in the chemical laboratory at the Ministry of Internal Affairs (MIA). That is not mentioned in the Review of Government Affairs for 1860 and 1861. However, that information appears in the writings by Dr. Lindenmayer during the Turkish bombardment of Belgrade on June 3–5, 1862, when a crisis headquarters was formed, the medical part of which comprised the chief of health, unassigned at the time, Dr. Emmerich Lindenmayer, and the Belgrade supervising physician, Dr. Jovan Mašin, who, among other things, had the task of organizing a medical facility and a hospital with 500 beds for the wounded. Then, with the knowledge of the Minister of Defence Monden, it was decided "that without hesitation and delay, a military pharmacy with a large stock should be established and that Josif Katrain, a well-known apothecary in Belgrade with a degree, should be positioned in it." That situation was later made official on August 28, 1862, when Katrain was appointed by the Prince's Decree as a temporary military apothecary in the Belgrade Military Hospital, a position where he would remain until his untimely death. After obtaining Serbian citizenship on April 1, 1865, he was appointed as an executive apothecary second class and promoted on December 1, 1871, to an apothecary first class. He brought order to the military pharmacy and can be *de facto* considered the first real educated and permanent Serbian military apothecary. Due to his early

Okretan i snalažljiv, za tadašnju čaršiju učen (govorio je srpski, grčki, vlaški, cincarski, turski, a „od časti nemecki") i u vojsci se dobro snalazio, što se vidi i iz službene ocene koju mu je dao pomoćnik načelnika srpskog saniteta dr Filip Taisić: „Dara je (prirodnog) dobrog, vrlo je bistar i s(h)vatljiv. U dužnosti je sposoban i vrlo upotrebljiv i pouzdan... empiričar je, vešt za ekspediciju lekova i vrlo dobro otpravlja posao pomoćnika lekarskog, u službi vrlo revnostan, tačan, pažljiv". To se vidi i iz odlikovanja koja je dobio: Takovski krst 5. reda, Srebrna medalja za revnosnu službu i Medalja Crvenog Krsta.

Umro je u Beogradu, doživevši da vidi slavu svoga sina jedinca kojim se ponosio [11–15].

Josif Katrain (Vinkovci, 11.02.1823 – Beograd, 16. juli 1872)

Josif Katrain rođen je u mnogočlanoj porodici (tri muška i dva ženska deteta) od oca Ivana, tesara i majke Katarine, rođene Presnek. Posle završene osnovne škole i šestorazredne gimnazije u rodnom mestu, završio je dvogodišnje studije farmacije, verovatno u Pešti ili Pragu. Njegovo ime u Srbiji se prvi put pominje u Državnom šematizmu za 1855. godinu kao ime prvog pomoćnika (drugi je bio Radosav Šimić) kod apotekara Pavla Ilića u apoteci Uprave varoši Beograda „Praviteljstvujuća apoteka" do 10. juna 1859. godine kada je prodata mr. farm. Teodoru Sekuliću, a Pavle Ivić prešao na mesto državnog kemika pete klase u hemijsku laboratoriju pri Ministarstvu unutrašnjih dela (MUD). U Šematizmima za 1860. godinu i 1861. godinu se to ne pominje. Međutim, ta informacija se pojavljuje u spisu dr Lindenmajera, u vreme turskog bombardovanja Beograda 3–5. juna 1862. godine, kada je obrazovan krizni štab, čiji medicinski deo su bili načelnik saniteta na raspoloženju dr Emerih Lindenmajer i beogradski fizikus dr Jovan Mašin koji je između ostalog imao zadatak da organizuje sanitet i bolnicu od 500 kreveta za ranjenike. Tada je, uz znanje vojnog ministra Mondena odlučeno „da se bez oklevanja i odugovlačenja obrazuje vojna apoteka sa velikim zalihama i da se u njoj postavi Josif Katrajn, dobro poznati u Beogradu diplomirani farmaceut". To stanje je kasnije ozvaničeno 28. avgusta 1862. godine kada je Katrain Knjaževim Ukazom postavljen za privremenog vojnog apotekara u beogradskoj vojnoj bolnici, mestu na kome će ostati do svoje prerane smrti. Po prelasku u srpsko državljanstvo 1. aprila 1865. godine postavljen je za dejstviteljnog apotekara druge klase, a unapređen 1. decembra 1871. godine za apotekara prve klase. Uveo je red u vojnoj apoteci, te se *de facto* može smatrati za prvog pravog školovanog i trajnog srpskog vojnog apotekara. Zbog prerane smrti, o njemu je sačuvano malo podataka, koji se, pojedinačno, nalaze na više strana. Čak i dr Vladan Đorđević, koji je, pišući svoje knjige o istoriji srpskog vojnog saniteta, imao na raspolaganju celu dotadašnju netaknutu vojnu arhivu, našao je o njemu samo jedan dokumenat.

death, little information about him has been preserved and can be individually found on several pages. Even Dr. Vladan Đorđević, who, when writing his books on the history of Serbian military health, had the entire previously untouched military archive at his disposal, found only one document about him.

According to the Catholic Church's Register of Deaths, he died after a short illness from "inflammation of the brain". He was married to Ana, born Šefer [16–23].

Mihailo Birg (Dunaföldvár, January 29, 1809‡ – Belgrade, exact date unknown, 1880/81)

No further family information is known, nor where Mihailo Birg completed his primary and secondary education. He completed his studies for the title of Master of Pharmacy in Pest in 1835, and after that, he worked as an apothecary in Szeged until he came to Serbia when he was already 55 years old.

He came to Serbia on November 8, 1864, and on November 20, he was appointed apothecary at the Military Hospital in Kragujevac. In 1873, he was transferred to the same duty in the Belgrade Military Hospital, where he worked until the end of his service in 1880, unassigned for the last two years. On November 30, 1875, he received the rank of second lieutenant and lieutenant in 1878 (according to the conduit list).

During the Serbian-Ottoman wars of 1876 and 1877/78, he continued working in the hospital. According to the official assessment given in his personal file in 1874 by Major Filip Tajsić, assistant to the head of health, he is "weak and silent because of his age, skilled and useful in pharmacy work. In his service, he is very zealous, punctual, durable, and diligent. He is not a good economist and does not know how to distribute his salary; because of this, he has deductions from his salary. He should be unassigned."

He spoke Serbian, Hungarian, German, and Latin. He was married and had one daughter [24–28].

Miloš Mihailović/Mihajlović (Novi Sad, December 22, 1822 – Kragujevac, November 17, 1887)

Miloš Mihailović obtained primary and secondary education in his hometown, Novi Sad, and received education in medical and pharmaceutical sciences in Vienna. Before he came to Serbia, he was an "apprentice in civic pharmacies".

He joined the Serbian military service as a foreign citizen on June 25, 1865. When he received Serbian citizenship after seven years, he was transferred to permanent service on November 20, 1872, as an apothecary's apprentice second class, and on May 1, 1875, as an apothecary's apprentice first class. In March 1876, when military ranks were introduced in the health department, he was promoted to the rank of lieutenant.

‡In Military Review for 1874, p. 171, it is written that he was born on January 7, 1807.

Prema katoličkoj crkvenoj knjizi umrlih, umro je posle kraćeg bolovanja od „zapaljenja mozga". Bio je oženjen Anom, rođenom Šefer [16–23].

Mihailo Birg (Dunafeldvar, 29. januar 1809§ – Beograd, tačan datum nepoznat 1880/81)

Nisu poznati bliži porodični podaci, niti gde je Mihailo Birg završio osnovno i srednje obrazovanje. Studije za zvanje magistra farmacije završio je u Pešti 1835. godine i posle toga je radio kao apotekar u Segedinu, sve do dolaska u Srbiju, kada je već imao 55 godina.

U Srbiju je došao 8. novembra 1864. godine i 20. novembra je bio postavljen za apotekara pri Vojnoj bolnici u Kragujevcu. Na istu dužnost u beogradskoj vojnoj bolnici premešten je 1873. godine i u njoj radio do kraja službe 1880. godine, pošto je poslednje dve godine bio na raspoloženju. Dobio je čin potporučnika 30. novembra 1875. godine, a 1878. godine čin poručnika (prema konduit-listi).

Za vreme srpsko-turskih ratova 1876. godine i 1877/78. ostao je na radu u bolnici. Prema službenoj oceni koju je u njegovom personalnom kartonu dao 1874. godine major dr Filip Tajsić, pomoćnik načelnika saniteta, on je „zbog starosti slab i ćutljiv, u apotekarskom poslu vešt i upotrebljiv. U službi je vrlo revnostan, tačan, izdržljiv, marljiv. Nije dobar ekonom i ne ume da raspoređuje platu, zbog ovoga mu se odbija od iste. Valjalo bi ga staviti u stanje pokoja."

Govorio je srpski, mađarski, nemački i latinski. Bio je oženjen i imao jednu ćerku [24–28].

Miloš Mihailović/Mihajlović (Novi Sad, 22. decembar 1822 – Kragujevac, 17. novembar 1887)

Miloš Mihailović u rodnom mestu Novom Sadu, stekao osnovno i srednje obrazovanje, a u Beču znanje medicinsko-farmaceutske nauke. Pre nego što je došao u Srbiju bio je „asistent u građanskim apotekama".

U srpsku ugovornu vojnu službu kao strani državljanin stupio je 25. juna 1865. godine. Kada je posle sedam godina dobio srpsko državljanstvo preveden je u stalnu službu 20. novembra 1872. godine u zvanju apotekarskog pomoćnika druge klase, a 1. maja 1875. godine u apotekarskog pomoćnika prve klase. Marta 1876. godine, kada su u sanitet uvedeni vojni činovi, preveden je u čin poručnika.

Prvih sedam godina je radio u beogradskoj Vojnoj bolnici, a zatim je premešten u kragujevačku vojnu bolnicu i u njoj ostao do penzije 1887. godine. Tamo je radio u miru i tokom srpsko-turskih ratova 1876. godine i 1877/78. godine, dok je u Srpsko-bugarskom ratu 1885. godine, iako već u godinama, bio apotekar u sanitetskoj četi pri zavojištu Šumadijske divizije. Odlikovan je Srebrnom medaljom za

§U Vojnom šematizmu za 1874, str. 171, piše da je rođen 7. januara 1807. godine.

For the first seven years, he worked in the Belgrade Military Hospital, and then, he was transferred to the Kragujevac Military Hospital and continued working there until his retirement in 1887. He worked there in peace and during the Serbian-Ottoman wars of 1876 and 1877/78, while in the Serbo-Bulgarian War of 1885, although already in his old age, he was an apothecary in the medical company for first-aid medical treatment of the Šumadija Division. He was awarded the Silver Medal for Zealous Service. In the official evaluation for 1874 given by the head of the Serbian military medical service, Lieutenant Colonel Dr. Karlo Beloni, it is written: "Strong and sturdy, capable of service in the field, reliable in his word. A good and accurate apothecary, punctual and diligent in his service. Gentle and polite to the younger ones, respectful to the equals, attentive and considerate to the older ones. In service, serious and useful, out of it, of pure character, very peaceful. Socializes with his equals. Deserves to be promoted."

He died, just retired.

In addition to the Serbian language, he spoke German, Hungarian, and Latin. Family information is unknown [29–39].

Mihailo Herman (Novi Sad, September 22, 1822 – Belgrade, after 1888)

Mihailo Herman was born to father Mihail and mother Elizabeth, born Gross. He completed primary school and a six-grade gymnasium in his hometown. He studied pharmacy at the University of Pest and obtained the title of Master of Pharmacy.

After graduating, he worked in pharmacies in Vienna and Pest. In 1859, as a military apothecary, he participated in Austria's war with the French and Italians on the Italian side.

He came to Serbia at the beginning of 1868, and on January 25, he was accepted into military service as a contract apothecary apprentice in the Belgrade Military Hospital. On November 30, 1875, he was promoted to the rank of second lieutenant and, as such, participated in the wars with Turkey in 1876 in the West-Moravian Division that fought on the western part of the front. In 1877/78, he served in the IV Field Hospital of the Moravian Corps when he was awarded the Silver Medal for Zealous Service. When the Great Niš Military Hospital was founded, he was transferred to work there. After the wars ended, he returned to his old service in the Belgrade Military Hospital. On May 21, 1881, he became a lieutenant. In the Serbian–Bulgaria War in 1885, he was an apothecary in the medical company for first-aid medical treatment of the Danube Division. After 1888, no information can be found on him in the Reviews of Government Affairs.

As for the official grades, he was given the first one in 1874 by Major Dr. Filip Tajsić, who says: "He is of ordinary talent, usable for the field service. Diligent and punctual in his service, although non-permanently and unwillingly. Dishonest towards his superiors and of a pliable and unstable character. He is serious in his service, but outside of it, he behaves irresponsibly towards his position, he is a bad economist, and he abuses medicines." Nevertheless, he was kept in the service. Two wars in which he participated have passed, and in 1879, Major Dr. Mihailo Marković, the future head of military health, wrote a new evaluation for him, saying: "He is of quiet nature, good in revnosnu službu. U službenoj oceni za 1874. godinu koju je dao načelnik srpskog vojnog saniteta potpukovnik dr Karlo Beloni piše: „Snažan i krepak, sposoban za službu u polju, pouzdan u svojoj reči. Dobar i tačan apotekar, u službi tačan i revnostan. Prema mlađima blag i uljudan, prema ravnima pristojan, prema starijima pažljiv i smotren. U službi ozbiljan i koristan, van nje karaktera čistog, vrlo miroljubiv. Druži se sa sebi ravnima. Zaslužuje da se unapredi."

Umro je tek penzionisan.

Pored srpskog govorio je nemački, mađarski i latinski. Porodični podaci nepoznati [29–39].

Mihailo Herman (Novi Sad, 22. septembar 1822 – Beograd, posle 1888)

Otac Mihaila Hermana bio je Mihail, a majka Elizabeta, rođena Gros. U rodnom mestu završio je osnovno i šestorazredno gimnazijsko obrazovanje. Studirao je farmaciju na peštanskom univerzitetu i stekao zvanje magistra farmacije.

Po diplomiranju je radio po apotekama u Beču i Pešti. Godine 1859. je kao vojni apotekar učestvovao u Italiji, u ratu Austrije sa Francuzima i Italijanima.

U Srbiju je došao početkom 1868. godine i već 25. januara primljen u vojnu službu kao ugovorni apotekarski pomoćnik u beogradsku vojnu bolnicu. U čin potporučnika unapređen je 30. novembra 1875. godine i potom učestvovao u ratovima sa Turskom 1876. godine u Zapadno-moravskoj diviziji koja se borila na zapadnom frontu. Zatim je 1877/78 služio u IV poljskoj bolnici Moravskog korpusa, kada je odlikovan Srebrnom medaljom za revnosnu službu. Pri osnivanju Velike niške vojne bolnice premešten je na rad u nju. Po završetku ratova vraćen je na staru službu u beogradsku vojnu bolnicu. Poručnik je postao 21. maja 1881. godine. U srpsko-bugarskom ratu 1885. godine bio je apotekar u sanitetskoj četi pri zavojištu Dunavske divizije. Posle 1888. godine ne nalazi se u šematizmima.

Što se tiče službenih ocena, u prvoj koju mu je dao 1874. godine major dr Filip Tajsić navodi se da je „običnog dara, upotrebljiv za poljsku službu. U službi je revnostan i tačan, no ne trajno i voljno, prema starešinama je neiskren, karaktera povodljivog i nestalnog. U službi je ozbiljan, a van nje se ponaša neodgovorno prema svome položaju, rđav je ekonom, pravi zloupotrebe sa lekovima." Ipak je ostavljen u službi. Prošla su dva rata u kojima je učestvovao i 1879. godine mu novu ocenu piše major dr Mihailo Marković, budući načelnik vojnog saniteta, koji kaže: „tihe naravi, dobar u struci, tačan i revnostan, radi sa voljom, učtiv, vrlo dobrog vladanja u i van službe, dobar ekonom. Zaslužuje preporuku!" Sličnu ocenu mu je dao 1880. godine i vojni apotekar kapetan prve klase dr Alojz E. Helih.

Bio je oženjen i bez dece [40–49].

his profession, punctual and zealous, works with will, polite, of very good behavior in and out of service, good economist. He deserves a recommendation!" A similar assessment was given to him in 1880 by the military apothecary Captain First Class, Dr. Alojz Helih.

He was married with no children [40–49].

Hristifor Dimitrijević (Novi Bečej, July 21, 1829 – Belgrade, November 9, 1896)

After completing elementary school in his hometown and a six-grade gymnasium in Novi Sad, Hristifor Dimitrijević went to Vienna to study pharmaceutics, where he completed the pharmaceutical course. After completing his education, he worked as a civil apothecary in various places.

He came to Serbia at the beginning of the war year of 1876 and entered the military medical service on March 10 of the same year as a contract second lieutenant. He kept that position until his death without changing his citizenship. His first job was in the Belgrade Military Hospital. At the beginning of the war with Turkey in 1876, he was the operator of the pharmacy depot in the Timok Division, for which we assume that it was in Zaječar. When Zaječar was abandoned, and the entire Active Army was reorganized after suffering losses into the Morava-Timok Army under the command of General Chernyaev, until the end of the war, Hristifor was assigned as an apothecary at the field hospital of the Lukovo Corps in the village of Krivi Vir, whose head of health was Major Dr. Stevan Nedok. In the 1877/78 war, he was an apothecary at the III Field Hospital of the Šumadija Corps, and after the armistice, he worked at the Great Niš Military Hospital. After the end of the war, from November 5, 1878, until 1887, he remained in the Military Hospital in Niš. During the Serbo-Bulgarian War in 1885, he stayed in Niš, first in the reserve medical company at the Supreme Command, and after the armistice, he returned to the Niš Military Hospital. In 1887/88, he had a break in service for eight months and eight days, and after that, until his death, he maintained the service in Belgrade, either in the Military Hospital or in the main warehouse of medical supplies. Judging by this, it seems that he died while still active in the service. For his participation in wars, he was awarded the Silver Medal for Zealous Service. On September 13, 1880, he was promoted to the rank of apothecary first class.

In the official evaluation from 1879, written by Captain Dr. Jovan Porubović, it is stated: "naturally gifted, physically strong, of open and venturesome character, and gentle nature. Capable of the position he is in, even for a higher one. He is very zealous in performing his duties, which comes from his own personality. He is considerate and attentive to the seniors, and towards the younger ones, he behaves like an elder. His behavior is serious and exemplary. He is a good economist. Deserves a higher rank".

He was educated [he spoke, read, and wrote in German, Latin, Vlach (Romanian), and Hungarian]. He was a widower with one female child [50–65].

Hristifor Dimitrijević (Novi Bečej, 21. juli 1829 – Beograd, 9. novembar 1896)

Posle završene osnovne škole u rodnom mestu i šestorazredne gimnazije u Novom Sadu, Hristifor Dimitrijević otišao je u Beč na studije farmaceutike i tamo „svršijo farmaceutski kurs". Posle završenog školovanja radio je kao građanski apotekar u raznim mestima.

U Srbiju je došao u početku ratne 1876. godine i stupio u vojni sanitet 10. marta iste godine, u svojstvu ugovornog potporučnika. U tome stanju ostaje sve do svoje smrti, ne menjajući državljanstvo. Prvo radno mesto bilo mu je u beogradskoj vojnoj bolnici. U početku rata sa Turskom 1876. godine bio je rukovalac apotekarskog depoa u Timočkoj diviziji (pretpostavljamo da je to bio Zaječar). Kada je Zaječar bio napušten, a celokupna dejstvujuća vojska posle pretrpljenih gubitaka reorganizovana u moravsko-timočku vojsku pod komandom generala Černjajeva, zatekao se i bio tu do kraja rata u selu Krivi vir na mestu apotekara poljske bolnice Lukovskog korpusa, čiji je načelnik saniteta bio major dr Stevan Nedok. U ratu 1877/78. godine bio je apotekar III poljske bolnice Šumadijskog korpusa, a po primirju je radio u Velikoj niškoj vojnoj bolnici. Posle završenog rata, od 5. novembra 1878. godine pa sve do 1887. godine ostao je u stalnoj niškoj vojnoj bolnici. U vreme srpsko-bugarskog rata 1885. godine ostao je u Nišu, prvo u rezervnoj sanitetskoj četi pri Vrhovnoj komandi, a po primirju se vratio u nišku vojnu bolnicu. Imao je prekid u službi osam meseci i osam dana 1887/88. godine, a posle toga, sve do smrti, bio je sa službom u Beogradu, bilo u vojnoj bolnici ili u Glavnom slagalištu sanitetskog materijala. Sudeći prema ovome, izgleda da je umro još aktivan u službi. Za učešće u ratovima odlikovan je Srebrnom medaljom za revnosnu službu. U zvanje apotekara prve klase preveden je 13. septembra 1880. godine.

U službenoj oceni iz 1879. godine, koju je pisao kapetan dr Jovan Porubović, stoji: „prirodnog dara običnog, telesno krepak, karaktera otvorenog i preduzimljivog, naravi blage. Sposoban za položaj u kome je, a i za veći. U vršenju službe vrlo je revnostan i to dolazi od njegove sopstvenosti. Prema starijima je smotren i pažljiv, a prema mlađima ponaša se starešinski. Vladanja je ozbiljnog i primernog. Ekonom je dobar. Zaslužuje veći čin".

Bio je obrazovan [govori, čita i piše nemački, latinski, vlaški (rumunski) i mađarski], udovac, sa jednim ženskim detetom [50–65].

Alojz E. Helih (Pođebradi, Češka, 7. juni 1843 – Beograd, 9. mart 1902)

Alojz E. Helih bio je najvažniji i najspremniji vojni apotekar srpske vojske XIX veka, njen glavni apotekar. Dospeo je, izuzetno, do ranga potpukovnika (viši intendant druge klase).

Njegov otac, Emanuel, bio je apotekar u mestu Pođebradi/Češka. Alojz je u rodnom mestu učio osnovnu školu i punu gimnaziju, a na Univerzitetu u Pragu završio je četvorosemestralno školovanje (1865/66) za magistra

Alojz E. Helih (Poděbrady, Czech Republic, June 7, 1843 – Belgrade, March 9, 1902)

The most important and prepared military apothecary of the Serbian Army of the 19th century was Alojz E. Helih, who was also the chief apothecary of the Serbian Army. Uncommonly, he reached the rank of lieutenant colonel (senior quartermaster second class).

His father, Emanuel, was an apothecary in Poděbrady, a town in the Czech Republic. Alojz completed elementary school and an eight-grade gymnasium in his hometown, and at the University of Prague, he studied for four semesters (1865/66) for a master's degree in pharmacy. Then, as an imperial scholarship holder, he spent two more years (1867 and 1868) training in the University Chemical Laboratory and became a chemist. Before coming to Serbia, he worked in Germany and Austria in pharmacies and chemical factories, among other things, in a factory for gunpowder production.

He came to Serbia following a competition at the beginning of 1872 and started working in the State Chemical Laboratory at MIA, of which he was also the director for a short time. On November 10, 1872, he transferred to the contract military service as an apothecary of the Belgrade Military Hospital and manager of the Main Pharmacy Depot. Until September 20, 1874, he was in service by contract as a foreign citizen. Uncommonly, before the usual duration period of seven years, he was granted Serbian citizenship on April 18, 1874, and became a permanently employed civilian in the Army. On May 1, 1875, according to the new Law on Army Organization, he was already promoted to the rank of medical captain first class, the highest rank for military apothecaries that existed in those times. On September 2, 1883, as a sign of honor and respect but contrary to the law, he was promoted to the rank of medical major, and on December 6, 1884, he received the title of senior quartermaster second class, which was equal to the rank of lieutenant colonel.

In 1884, using his earlier experiences from a gunpowder factory in Austria, he examined the purity of the saltpeter produced in the military gunpowder factories in Stragari and Obilićevo near Kruševac. He determined the impurities, simultaneously devising a procedure for their removal. He checked his results at the University Chemistry Laboratory in Stuttgart, which he announced in a published paper in a chemical magazine. That contributed to the improved properties of gunpowder, primarily to its chemical stability, because until then, it was chemically unstable, which led to spontaneous self-ignitions.

From the position he was in, he tried to ensure that the Serbian Army was supplied as much as possible with medical supplies and medicines, which was not always easy due to the poverty of the young country. With the obtained knowledge and a lot of will and energy, he managed the Serbian pharmacy throughout the three wars that Serbia fought in the 19th century, and many wounded or sick people had to thank him for saving their lives. His enormous effort is best expressed by the words of Dr. Vladan Đorđević during the Serbo-Bulgarian War in 1885: "On the very first day (of taking office as the Chief of Medical Services of the Supreme Command), I farmacije. Potom se kao carski stipendista još dve godine (1867. i 1868) usavršavao u Univerzitetskoj hemijskoj laboratoriji i postao diplomirani hemičar. Do dolaska u Srbiju radio je u Nemačkoj i Austriji u apotekama i hemijskim fabrikama, između ostalog i u fabrici za proizvodnju baruta.

U Srbiju je došao po konkursu početkom 1872. godine i započeo rad u Državnoj hemijskoj laboratoriji pri MUD, čiji je kraće vreme bio i upravnik. Desetog novembra 1872. godine prešao je u ugovornu vojnu službu na mesto apotekara Beogradske vojne bolnice i upravnika Glavnog apotekarskog depoa. Do 20. septembra 1874. godine bio je, kao strani državljanin, u ugovornoj službi, a pošto je, izuzetno pre sedmogodišnjeg roka, 18. aprila te godine prihvaćen u srpsko državljanstvo, postao je „dejstvitelni" (stalni) vojni službenik. Već 1. maja 1875. godine je, po novom Zakonu o ustrojstvu vojske, unapređen u čin sanitetskog kapetana prve klase, tada najviši čin za vojne apotekare. Zatim, 2. septembra 1883. godine, u znak počasti i poštovanja, mimo zakona, unapređen je u čin sanitetskog majora, a 6. decembra 1884. godine dobio je zvanje višeg intendanta druge klase, što je bilo ravno činu potpukovnika.

Godine 1884, koristeći svoja ranija iskustva iz fabrike baruta u Austriji je ispitivao čistoću šalitre koja se proizvodila u vojnim fabrikama baruta u Stragarima i Obilićevu kod Kruševca i utvrdio nečistoće, smislivši istovremeno postupak za njihovo uklanjanje. Svoj rezultat je proverio u Univerzitetskoj hemijskoj laboratoriji u Štutgartu, što je saopštio i u objavljenom radu u poboljšanju časopisa za hemiju. Time je doprineo poboljšanju svojstvava baruta, pre svega njegovoj hemijskoj postojanosti. Do tada je barut bio hemijski nestabilan, što je dovodilo do spontanih samozapaljivanja.

Sa položaja na kome se nalazio, Alojz E. Helih se trudio da srpska vojska bude što bolje snabdevena apotekar-skim i medicinskim materijalom i lekovima, što, zbog siromaštva mlade države nije bilo uvek lako. Imajući znanje, volju i energiju vodio je srpsko apotekarstvo u sva tri rata koja je Srbija vodila u XIX veku i, zahvaljujući njemu, mnogim ranjenicima i bolesnicima su sačuvani životi. Koliko se on trudio, najbolje govore reči dr Vladana Đorđevića za vreme Srpsko-bugarskog rata 1885. godine: „Odmah prvog dana (stupanja na dužnost načelnika saniteta Vrhovne Komande) potražio sam podatke kako stoji vojska sa lekovima, zavojnim priborima, instrumentima i ostalom opremom sanitetskih trupa... Istoga dana (19. septembra 1885. godine) dobio sam od majora Heliha ovakav izveštaj: vojne apoteke u Nišu i Kragujevcu snabdevene su tako da u mirno doba imaju dosta za šest meseci. Osim neznatne nabavke pogdekojeg leka ima u područnom mi glavnom apotekarskog slagalištu dovoljno lekova da se sve poljske apoteke napune i da još beogradska vojna apoteka ne oskudeva. Držim da sada imamo u zemlji dovoljno lekova da podmirimo sve potrebe naše vojske i u vanredom stanju najmanje za dva meseca." Na ovo dr Vladan zaključuje da se za ovakvo stanje „ima zahvaliti jedino

looked for information on how the Army was doing with medicines, bandages, instruments, and other broken equipment of the medical troops... On the same day (September 19, 1885), I received the following report from Major Helih: the military pharmacies in Niš and Kragujevac are supplied so much that there would be enough supplies for six months in peacetime. Apart from the insignificant procurement of some medicine, there is enough medicine in our regional main pharmacy warehouse to fill all field pharmacies, and, even then, the Belgrade military pharmacy would not be lacking any supplies. I believe that we now have enough medicine in the country to meet all the needs of our army even in a state of emergency for at least two months." On this, Dr. Vladan concluded that for this state of affairs "...we have to thank only the foresight and energy of the chief military apothecary who, in peacetime, did not forget the first rule of every military administration – it is in peacetime that war is prepared for". Here is the assessment given to him in 1880 by the director of the Belgrade Military Hospital, Lieutenant Colonel Dr. Josif Holec: "Very gifted and bright, with an open character, venturesome, quiet. Healthy, agile, and persistent. A highly educated apothecary, as well as a chemist and microscopist, follows the progress of those sciences. A very reliable and accurate administrator and a caring teacher to his younger associates. Excellent in every respect (in the service), performs all tasks with great will, carefully and accurately. Very respectful and polite towards the elderly, gentle and moderately strict towards the younger ones. In general, he is very pleasant, showing everywhere a very large level of acquired education. He is very modest, but he lives decently. According to these virtues, which distinguished him in his difficult and long-lasting service, it is just to say that he deserves to be promoted."

Alojz E. Helih was the chief apothecary of the Serbian Army, a member of the Military Medical Committee until his death, and a teacher to many generations of military apothecaries. He died in active service, and the following year, his only son, Lieutenant Dr. Jaroslav Helih, a Serbian military scholar who brilliantly completed his medical studies in Prague, died at a very young age (Belgrade, December 1, 1874 – Zaječar, November 15, 1903). Besides his son, with his wife Božena, born Bouček, he also had a daughter, Ludmila (Belgrade, September 27, 1876 – Belgrade, date of death unknown), later known as Ruža (her baptized name perhaps), who became the director (1924–1931) of the Belgrade "School for Nurses" of the Red Cross Society.

Lieutenant Colonel Helih was the holder of many military decorations: Cross of Takovo V Order (1878), IV Order (1889), III Order (1893), Order of Saint Sava III Order (1894), Gold Medal for Zealous Service (1895), Cross of the Society of the Red Cross (1878), Memorials of the Wars 1876, 1877/78, 1885 [66-84].

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uviđavnosti i energiji glavnog vojnog apotekara koji ni u najdubljem miru ne zaboravljaše na prvo pravilo svake vojne administracije, a to je da se baš u mirnodopsko vreme valja spremati za rat". Evo ocene koju mu je 1880. godine dao upravnik beogradske vojne bolnice, potpukovnik dr Josif Holec: „Vrlo darovit i vrlo bistar, otvorenog karaktera, preduzimljiv, tih. Zdrav, okretan i izdržljiv. Vrlo izobražen apotekar, kao i hemičar i mikroskopičar, prati napredak ovih nauka. Vrlo pouzdan i tačan administrator, brižljiv učitelj svojih mlađih. (U službi) u svakom pogledu odličan, vrši sve poslove sa osobitom voljom, smotreno i tačno. Prema starijima vrlo učtiv i pristojan, prema mlađima blag, umereno strog. Uopšte je vrlo prijatan drug, pokazujući svud vrlo veliki kapital izobraženja. Vrlo je skroman, no živi pristojno. Prema ovakvim vrlinama, kojima se u teškoj i višegodišnjoj službi odlikovao, pravedno je da već zasužuje unapređenje."

Alojz E. Helih bio je glavni apotekar srpske vojske, član Vojno-sanitetskog komiteta do smrti, učitelj mnogih generacija vojnih apotekara. Umro je u aktivnoj službi, a naredne godine umro je njegov sin jedinac, mladi poručnik dr Jaroslav Helih (Beograd, 1. decembar 1874 – Zaječar, 15. novembar 1903), srpski vojni stipendista, koji je briljantno završio studije medicine u Pragu. Pored sina, sa suprugom Boženom, rođenom Bouček imao je i ćerku Ludmilu (Beograd, 27. septembar 1876 – Beograd, datum smrti nepoznat), kasnije poznatu kao Ruža (moguće pokršteno ime), koja je kasnije (1924–1931) bila direktorka beogradske „Škole za nudilje" Društva Crvenog Krsta.

Potpukovnik Helih bio je nosilac mnogih vojnih odličja: Takovski krst V reda (1878), IV reda (1889), III reda (1893), Orden Svetog Save III reda (1894), Zlatna medalja za revnosnu službu (1895), Krst Društva Crvenog Krsta (1878), Spomenice ratova 1876. godine, 1877/78. godine, 1885. godine [66–84].

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BOOK REVIEW/PRIKAZ KNJIGE

Fundamentals of Clinical Pharmacology

Title: Fundamentals of Clinical Pharmacology
Original title: Osnovi kliničke farmakologije (Serbian)
Authors/Autori: Viktorija Dragojević Simić, Silva Dobrić, Momir Mikov, Ranko Škrbić
Publisher/Izdavač: Medija centar „Odbrana“, Beograd
Year/Godina izdanja: 2021
ISBN: 978-86-335-0741-7

The textbook *Fundamentals of Clinical Pharmacology* was edited and written by Professor Viktorija Dragojević Simić, Professor Silva Dobrić, Professor Momir Mikov, and Professor Ranko Škrbić, with as many as 20 co-authors from the Faculty of Medicine of the Military Medical Academy of the University of Defence in Belgrade, the Faculty of Medicine in Novi Sad, the University of Novi Sad, and the Faculty of Medicine in Banja Luka, University of Banja Luka.

It was necessary to upgrade and enrich the knowledge of drugs acquired by the medical students during the “Pharmacology” course with the content necessary for the everyday clinical practice of future medical doctors. In fact, an effort was made by the authors to present clinical pharmacology comprehensively, taking into account the fact that it is a modern interdisciplinary branch of medicine characterized by intensive development. With this textbook, the authors tried to rectify the insufficient representation of clinical pharmacology literature in the Serbian language.

However, as is usual in a textbook like this, the authors first dealt with the development of this discipline in the period of the existing Socialist Federal Republic of Yugoslavia and its scope in Serbia, mentioning those who should be given credit mostly for developing clinical pharmacology.

Chapters dealing with clinical pharmacokinetics, clinical importance of drug pharmacodynamics, good laboratory practice in preclinical trials, and drug interactions form a rounded whole. They represent topics that medical students have already encountered during the “Pharmacology” course but are now presented through a new clinical approach.

Considering the importance that clinical trials of new drugs have for the individual patient, but also for the wider social community, as well as the relevance of the topic, the authors have dealt with it comprehensively by including his-

Udžbenik pod nazivom „Osnovi kliničke farmakologije“, uredili su i napisali profesor dr Viktorija Dragojević-Simić, profesor dr Silva Dobrić, profesor dr Momir Mikov i profesor dr Ranko Škrbić, sa još čak 20 koautora sa Medicinskog fakulteta Vojnomedicinske akademije Univerziteta odbrane u Beogradu, Medicinskog fakulteta Novi Sad Univerziteta u Novom Sadu i Medicinskog fakulteta Univerziteta u Banja Luci.

Bilo je potrebno da se znanje o lekovima koje su studenti usvojili u okviru predmeta „Farmakologija“ nadogradi i obogati sadržajem koji je neophodan za svakodnevni klinički rad budućih lekara. Zapravo, nastojalo se da se klinička farmakologija predstavi sveobuhvatno, uzimajući u obzir činjenicu da se radi o savremenoj interdisciplinarnoj grani medicine, koju karakteriše intenzivan razvoj. Ovim udžbenikom autori su pokušali da isprave nedovoljnu zastupljenost literature za kliničku farmakologiju na srpskom jeziku.

Ipak, kako je uobičajeno, autori su se najpre bavili razvojem te discipline u periodu postojanja Socijalističke Federativne Republike Jugoslavije i njenim dometima u Srbiji, pominjući one ličnosti kojima treba pripisati najviše zasluga za razvoj kliničke farmakologije.

Poglavlja o kliničkoj farmakokinetici, kliničkom značaju farmakodinamike lekova, dobroj laboratorijskoj praksi u pretkliničkim ispitivanjima i interakcijama lekova čine jednu zaokruženu celinu. Ova poglavlja predstavljaju teme sa kojima su se studenti medicine već susretali na predmetu „Farmakologija“, ali su predstavljene kroz nov, klinički pristup.

S obzirom na značaj koji klinička ispitivanja novih lekova imaju za pacijenta – pojedinca, ali i za širu društvenu zajednicu, kao i na značaj teme, autori su se njom bavili

tory, methodology, ethics, statistics, and especially good clinical practice in clinical trials, as an internationally accepted standard that guides us to properly conduct studies involving humans.

The chapters that refer to the branches of clinical pharmacology that have been known for a long time, both globally and in our country, are the ones dealing with pharmacovigilance, pharmacoepidemiology, and pharmacoeconomics. On the other hand, the chapters on the implementation of the principles of pharmacogenomics in modern pharmacotherapy refer to both current and future opportunities in the realization of the implementation of personalized medicine in everyday medical practice.

Important topics, such as generic, substandard and falsified drugs, biotechnological drugs and biosimilars, as well as the ones concerning clinical trials proving the biological equivalence of investigated drugs, are also presented with contemporary knowledge.

Other essential topics, such as those on national policy of drugs and lists of medicines, as well as pharmacotherapy in special clinical states and age groups, are also covered in the first part of the textbook. Chapters on herbal and homeopathic medicines, over-the-counter drugs, and medical devices, which are also included, should inform future medical doctors about topics they are probably not yet sufficiently familiar with.

As it is very important for medical students to accept the concept of rational use of drugs as well as the currently generally accepted position that medicine must be based on evidence, the authors also dealt with these topics. In the textbook, the authors also discuss practical advice on how to obtain pharmacotherapeutic aspects of the medical history from patients, which is completely neglected and insufficiently developed in our clinical practice.

Most of the texts are accompanied by tables and graphs, and after each of them, a list of contemporary literature is attached. The literature often features authors' own publications, among others, speaking in favor of their long-term work and rich experience in the field of clinical pharmacology.

The second part of the book presents more than 30 clinical cases, such as examples of rational drug prescribing, including acute drug poisoning. They were chosen primarily according to their frequency and importance for the medical doctor in primary health care or as additional practical clarification of certain topics that were already covered in the first part of the textbook. These texts are also accompanied by appropriate additional literature.

In the third part of the textbook, five examples of pharmacokinetic tasks are given since students should gain insight into the possibility of calculating and applying the pharmacokinetic parameters of drugs in real-life clinical situations.

This textbook is the result of the contribution of a large number of authors who teach clinical pharmacology as a part of undergraduate and postgraduate education, including health specialization. It is a significant contribution to the expert literature in this field in the Serbian language. Due to this, it is adapted to the curriculum that students should mas-

sveobuhvatno, uključujući istoriju, metodologiju, etiku, statistiku, a posebno dobru kliničku praksu u kliničkim ispitivanjima, kao međunarodno prihvaćenom standardu koji nas upućuje na pravilno izvođenje studija koje uključuju ljude.

Poglavlja koja se odnose na one grane kliničke farmakologije koje su već dugo poznate u svetu, pa i kod nas, su ona koja se bave farmakovigilancom, farmakoepidemiologijom i farmakoekonomijom. S druge strane, poglavlja o implementaciji principa farmakogenomike u savremenu farmakoterapiju ukazuju i na sadašnje i buduće mogućnosti da se realizuje težnja za implementacijom personalizovane medicine u svakodnevnu lekarsku praksu.

Značajne teme, kao što su generički, supstandardni i falsifikovani lekovi, biotehnološki lekovi i biosimilari, kao i koncept paralelnosti lekova i ispitivanja koja dokazuju biološku ekvivalentnost ispitivanih lekova, takođe su savremeno obrađene i prikazane.

U prvom delu udžbenika obrađene su i druge bitne teme, poput onih o nacionalnoj politici lekova i listama lekova, kao i farmakoterapiji u posebnim kliničkim stanjima i uzrasnim grupama. Poglavlja o biljnim i homeopatskim lekovima, lekovima bez recepta i medicinskim sredstvima, koja su takođe uključena, trebalo bi da informišu buduće lekare o temama sa kojima verovatno još uvek nisu dovoljno upoznati.

Kako je za studente medicine veoma bitno da još u toku studija prihvate koncept racionalne primene lekova i sada opšteprihvaćeni stav da medicina mora biti zasnovana na dokazima, autori su se bavili i tim temama. Autori su se u ovom udžbeniku takođe bavili i praktičnim savetima o tome kako uzeti farmakoterapijsku anamnezu, što je u potpunosti zapostavljeno i nedovoljno razvijeno u našoj kliničkoj praksi.

Većina tekstova je obogaćena tabelama i grafikonima, a uz svaki je dat i popis savremene literature, u kome su često navedene i sopstvene publikacije autora, što govori o njihovom dugogodišnjem radu i bogatom iskustvu u oblasti kliničke farmakologije.

U drugom delu udžbenika dat je prikaz više od 30 kliničkih slučajeva, odnosno primera racionalnog propisivanja lekova, uključujući i akutna trovanja lekovima. Oni su birani prevashodno prema njihovoj učestalosti i značaju za lekara u primarnoj zdravstvenoj zaštiti ili kao dodatno praktično objašnjenje pojedinih tema koje su obrađene u prvom delu knjige. Ti tekstovi su takođe propraćeni odgovarajućom dodatnom literaturom.

U trećem delu udžbenika dato je pet primera zadataka iz farmakokinetike, kako bi studenti dobili uvid u mogućnost izračunavanja i primene farmakokinetskih parametara lekova u realnim kliničkim situacijama.

Ovaj udžbenik je plod rada velikog broja autora koji predaju kliničku farmakologiju u okviru dodiplomskih i poslediplomskih vidova edukacije, uključujući i zdravstvenu specijalizaciju. On predstavlja značajan doprinos nastavnoj literaturi iz te oblasti na srpskom jeziku. Zbog svega navedenog, prilagođen je nastavnom planu i programu koje studenti treba da savladaju kroz predmet „Klinička

ter through the “Clinical Pharmacology” course at all three Medical Faculties mentioned at the beginning of the text. However, the textbook can also serve as a starting point for the acquisition of new knowledge for medical doctors in post-graduate education in this field, as well as for other medical specialists who want to refresh and supplement their knowledge on some of the topics covered in this textbook.

farmakologija“ na sva tri Medicinska fakulteta navedena na početku teksta. Međutim, ovaj udžbenik umnogome može poslužiti kao polazna osnova za sticanje novih znanja i lekarima na poslediplomskim vidovima edukacije iz ove oblasti, ali i drugim specijalistima koji žele da osveže i dopune svoje znanje o nekoj od tema kojima se bavi ova knjiga.

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Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi **– Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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