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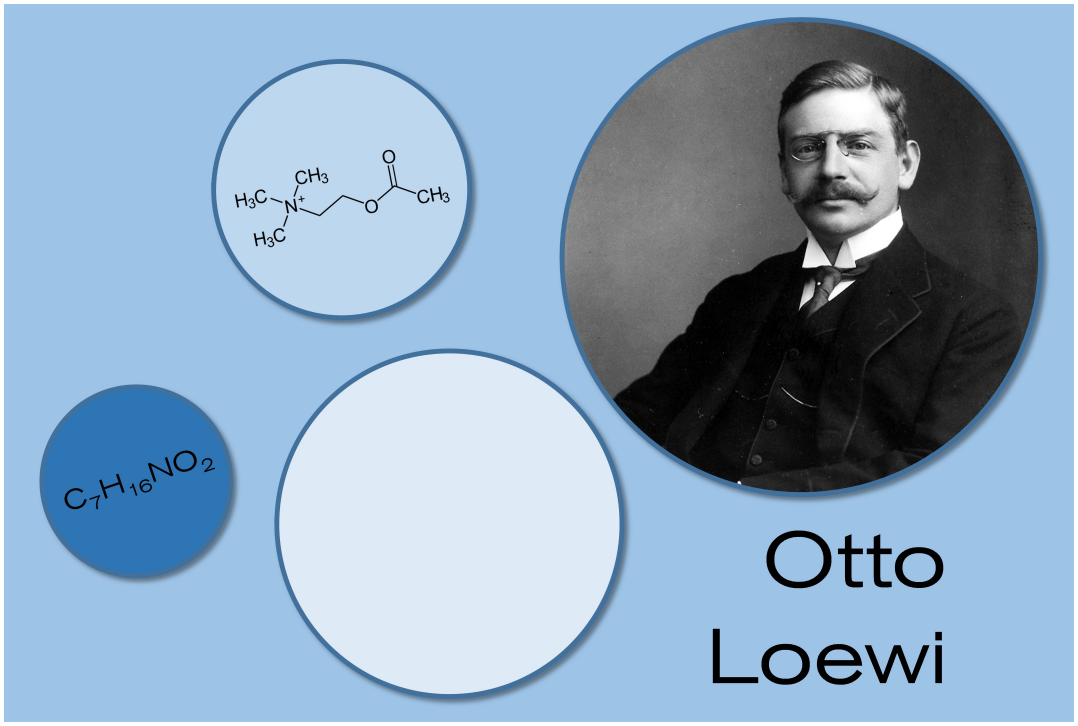

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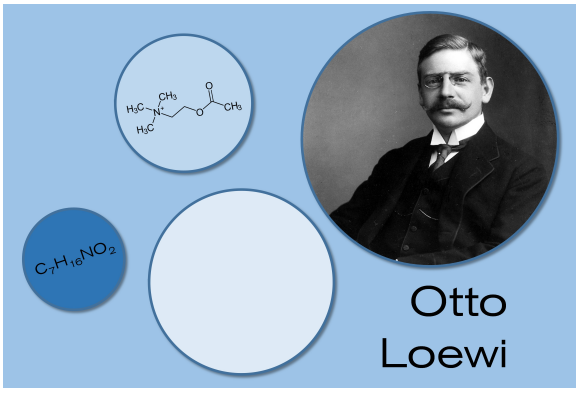
CONTENTS / SADRŽAJ

CASE REPORTS / KAZUISTIKA



Oto Levi (3. jun 1873. – 25. decembar 1961), čija se 150-ta godišnjica rođenja obeležava ove godine, bio je austrijski lekar i farmakolog koji je otkrio ulogu acetilholina kao endogenog neurotransmitera. Za svoje otkriće dobio je Nobelovu nagradu za fiziologiju ili medicinu (1936. godine), koju je podelio sa Henri Dejlom. U čuvenom eksperimentu na srčanom mišiću žabe, izvedenom 1921. godine, pokazao je postojanje do tada nepoznate solubilne supstance, koju je nazvao „vagustof“ (kombinacija imena nerva i nemačke reči za supstancu), jer je oslobađa *nervus vagus*, a koja utiče na broj otkucaja srca u odsustvu električne stimulacije. Danas znamo da je ova supstanca acetilholin, a Levijev eksperiment bio je temelj otkrića da se komunikacija među nervnim ćelijama odvija posredstvom hemijskih „glasnika” – neurotransmitera.

Otto Loewi (3 June 1873 – 25 December 1961), whose 150th birth anniversary is celebrated this year, was an Austrian physician and pharmacologist who discovered the role of acetylcholine as an endogenous neurotransmitter. For his discovery, Loewi received the Nobel Prize in Physiology or Medicine in 1936, which he shared with Henry Dale. In the famous experiment on the frog's heart muscle, performed in 1921, Loewi demonstrated the existence of a previously unknown soluble substance, which he called "vagusstoff" (a combination of the name of the nerve and the German word for the substance), because it is released by the *nervus vagus*, and which affects the number of heartbeats in the absence of electrical stimulation. Today we know that this substance is acetylcholine, and Loewi's experiment was the foundation of the discovery that communication between nerve cells takes place through chemical "messengers" – neurotransmitters.

G E N E R A L R E V I E W

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Psychotherapy in psychiatry: subspecialization or integration?

Psihoterapija u psihijatriji: subspecijalizacija ili integracija?

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Key words:
education; psychiatry; psychotherapy; specialization; serbia.

Ključne reči:
obrazovanje; psihijatrija; psihoterapija; specijalizacije, medicinske; srbija.

Introduction

A clearer understanding of the relationship between psychiatry and psychotherapy, both in everyday practice and education, is still needed today. The origin and development of psychoanalysis at the beginning of the twentieth century and its expansion following the Second World War, on the one hand, and immensely accelerated progress in the neurosciences and biological psychiatry started by the pharmaceutical industries, on the other, led to the separation of psychopharmacological treatment and psychotherapy. Economic pressure from insurance groups, with their requests for rapid improvement and prompt treatment for mental health problems, thus minimizing hospital stays, additionally led to favoring pharmacotherapy. All this brought up many questions regarding the relationship between psychiatry and psychotherapy. Many of these questions are yet to be answered. Today, we find ourselves wondering about the role and place of psychotherapy as a therapeutic method in psychiatry, and how both future and current psychiatrists should be educated during their training [1].

The paradox is present because, at the time of great research opportunities and the potential of science to understand the complexity of the mind-brain relationship and thus rise above the artificially created Cartesian dualism, psychiatry is becoming increasingly reductionist [2]. In favor of this, Gabbard [3] notes that psychotherapy considers the treatment of "psychologically caused" disorders, while medications used for treating these disorders affect the brain. In this way, a simplified dualism neglects that psychotherapy creates its impact by changing the brain and that the mind is the result of brain activity [3–5]. Recent papers discuss this issue in more depth. Nobel laureate Kandel [6] noted earlier that psychotherapy could be seen as a biological treatment, creating a parallel between psychotherapy and his research on marine molluscs *Aplysia californica*, which shows that synaptic connections can change indefinitely and increase the expression of genes when learning takes hold, which also happens in a successfully conducted psychotherapy. Some studies have confirmed that psychotherapy as a method of treatment in psychiatry needs a wider context and a more in-depth understanding. The role of psychotherapy in psychiatry should be assessed critically in many different aspects since the separation of these two might lessen not only the possibility of quality treatment for our patients but also the possibility for future psychiatrists to obtain complete training and education.

The aim of this paper was to systematically describe psychotherapy as an inseparable part of basic psychiatry training and keep the subspecialty possibility open.

This paper represents a review of the interaction, firstly through the history of psychiatric education, then through current integrative models, but also accentuates the importance of accepting the possibility of having psychotherapy as a psychiatric subspecialty (fellowship).

We shall begin with a historical review of the relationship as the base for understanding the origin of the artificial dichotomy of the mind-brain [3, 4] and the antagonism of biologically oriented psychiatrists towards psychotherapy.

Historical retrospective

The place and role of psychotherapy in psychiatry discussion starts during the first half of the twentieth century with the beginning of psychoanalysis and revolutionary

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breakthroughs in understanding mental functioning. This new therapeutic method based on free association and careful listening to the patients in a different way, which allows it to be interpreted as an integral part of psychoanalytic work, showed positive results and uncovered a new method of treatment [7]. Psychoanalysis provided many new ideas: knowledge about unconscious mental processes, psychic determinism, infantile sexuality, and possibly most important of all, uncovering the irrationality of human motivations [7]. In this way, psychoanalysis inspired the progress of the contemporary psychiatry of the time. Sigmund Freud's stay in America at the beginning of the twentieth century, along with the years after the Second World War when many European psychoanalysts emigrated to the more open United States (US) society, created the conditions for the development of psychoanalysis in America [8].

Kandel [7] suggested that medicine in that period transformed from a practical trade to molecular biology. Yet, at the same time, psychiatry transformed from a medical discipline to a therapeutic art. It is a surprising fact that during the 50s and 60s, academic centers in the US of America (USA) saw a shift from the biologically-based view of psychiatry to the more socially and psychoanalytically based context, lessening the focus on the brain as the organ of mental activity [6, 7]. The development of psychoanalytic psychiatry does not stop here, according to Kandel [7]. Instead, the development is spreading to different medical disorders that, prior to this type of treatment, did not respond to the pharmacological treatments mostly used in the 40s; thus, the basis of psychosomatic medicine was created. The reach of psychoanalytic therapy spread gradually yet considerably to almost all mental disorders, including general psychoses, schizophrenia, and clinical depression [6, 9].

In this way, the focus of the work, but also psychiatric training, shifted to psychoanalytic psychiatry. Moving from descriptive psychiatry of the pre-Second World War period to psychoanalysis undoubtedly proved beneficial to clinical insights through stronger explanations for observed phenomena; however, it did veer psychiatry away from the breakthroughs of biology and experimental medicine [6]. This situation does not present a good basis for the future position of psychotherapy in psychiatry as a *de facto* medical discipline. That is the beginning of the "seesaw" between biological psychiatry and psychotherapy, a continual shift that is more or less present today as well.

This deviation from biology is the result of the lack of concrete, in-depth knowledge about the brain at that time and also the result of the dominant belief that various mental functions could not be localized in specific regions of the brain and that many more mental functions are diffusely exhibited in the cortex [6]. In this way, the role of psychoanalysis as a psychotherapy method was the result of a number of conditions contributing to this dominance. Both psychiatry and psychology benefited from this separation, developing systematic definitions of behavior from then-unknown correlations with neuron mechanisms. Furthermore, the presence of psychoanalysis in psychiatry contributed to the greater focus on the human side of the interactions with the patient, reducing the stigma associated with the previous period. By 1960, psychoanalytically oriented psychiatry became the main model for understanding all mental and also some physical disorders [9].

Psychoanalysts failed to overcome the shortcomings of psychoanalysis through experimental research, given the role they had in psychiatry as a whole. In this way, psychoanalysis experienced a downfall of sorts, which influenced the entire psychiatry, discouraging new ways of thinking and affecting the quality of psychiatric training. The function of the specialization programs was not to develop good psychiatrists but rather good therapists who could empathize with their patients and their life issues [9].

Biological revolution in psychiatry

From 1950 to 1960, the years were marked by the development of psychopharmaceutic drugs, firstly chlorpromazine, then antidepressants (isoniazid, iproniazid, imipramine), then chlordiazepoxide, all of which contributed to a second revolution in the field of psychiatry [10]. However, the political dominance of psychoanalysis in psychotherapeutic training had several decades of influence at that time, and balancing that with these breakthroughs proved to be difficult. Several influential and critically oriented researchers of the succeeding era of psychiatry spoke of their personal experience during their psychiatry training, how their mentors would often say that the medication serves to lessen the anxiety of the doctor, not the patient. However, the middle of the 1970s showed a great need for understanding the mechanisms of psychopharmaceutic treatments instead of purely clinical observation and patient behavior, and so a new cycle began [9].

The development of psychopharmacology has, apart from the undoubtedly positive changes seen in the treatment of psychiatric patients due to it, pushed aside the important characteristics of psychotherapy. Most of all, in lowering the attention given to psychodynamic and developmental factors influencing psychopathology, mental disorders became a disease of the brain or a "chemical imbalance" in the eyes of the public and many psychiatrists. Changes in the relationship between health insurance toward psychotherapy have also had an impact on the programs of psychiatric specialization, as psychiatric disorders are becoming more identified with a biological or medical model and less with a biopsychosocial one. Because of this, it is presumed that, should this trend continue, psychiatry will lose its essence – humanism [11].

Due to the lack of time and changes in training and education, specialists in psychiatry have less opportunity to learn about the "time-dependent" elements of psychiatry: the capability of empathetic listening, development of a therapeutic alliance, working with resistance to therapy, understanding of psychodynamics, recognizing transfer phenomena, and where and how to provide interpretations [11].

To answer our questions and dilemmas related to the model of psychotherapeutic education and training in psychiatry and the dilemma of integration in the context of

the specialization of psychiatry as a model of subspecialization, as it was for years in Serbia, as it has been in recent years in the United Kingdom (UK), and as it currently is considered in the USA, we will focus on two models: European and American.

European model of psychiatric integration of psychotherapy

In international circles, after the rise of biological psychiatry, the reintegration of psychotherapy in psychiatry went in the following way. In 1958, the Union of European Medical Specialties (UEMS) was established, and after more than thirty years, in 1991, the Section for Psychiatry was formed. The European Forum for all Psychiatric Trainees in Utrecht was established after that with the idea of aligning knowledge through the mutual exchange of ideas and training throughout Europe, in order to aid organizations in individual nations [12–14]. They formed guidelines important for psychotherapeutic training in psychiatry. It was emphasized that basic training must include supervision of clinical practice, which would be supervised by qualified psychotherapists. Next, it was emphasized that in theoretical training, different areas of psychotherapy must be included, while skills are gained mostly in individual areas of psychotherapy. After this education, psychiatrists must be knowledgeable in other forms of psychotherapy as it would allow them to refer their patients to a specialized psychotherapist. Finally, a personal psychotherapeutic experience would be an important component in training, so programs of training for future psychiatrists should have it integrated into their residency training [1, 14].

Section for Psychiatry UEMS recommends to the national bodies that psychotherapy be seen as an integral part of training in psychiatry and that they are to be responsible for establishing a system to finance psychotherapeutic training, as it already is the case with other forms of training in psychiatry. The reason behind that is the fact that psychotherapeutic training would improve the clinical practice of psychiatrists [1, 13].

The theory of psychotherapy is a part of the graduate program and includes at least psychodynamic and cognitive-behavioral theory. Other theories can be included once they are scientifically confirmed. There is a predefined number of hrs of theoretical training, research methodologies, and individual psychotherapeutic cases supervised with a predefined number of hrs. Psychotherapy training (theory and supervision) could be individual or group. Lecturers are obliged to have the training in psychotherapy completed, and the training must be recognized by the national body. Since the training is evidently for psychotherapy in psychiatry, the head of the program has to be a psychiatrist [13].

One of the main missions of the Section for Psychiatry is the implementation of comprehensive knowledge of individual programs in the countries of the European Union in order to align psychiatric training. For obtaining more specific information on the training programs, it was decided that different areas of psychiatry must be looked at in the same way as the three-dimensional approach to psychiatry (psychology, sociology, and biology), which was present in member countries [12, 13].

The Section for Psychiatry UEMS, in Edinburgh, on April 2, 2004, defined psychiatry as a biopsychosocial discipline and acknowledged psychotherapy within this framework. It was defined as a psychological intervention that is structured, focused, and grounded on "evidence-based" medicine. They set it in the center of psychiatric disorders treatment, based on analysis of training [14–16]. According to them, the three theories of psychotherapy used in psychiatry are psychodynamic, behavioral, and systemic [17, 18].

Section for Psychiatry recognizes the following psychotherapeutic components of psychiatric training in Europe – a defined number of psychotherapy cases for clinical experience, a minimum of 120 hrs of theoretical training, and a minimum of 100 hrs of case supervision. That being said, the supervisors ought to be qualified, personal therapy is highly recommended, and training should be publicly funded [13, 17, 19].

USA model of psychiatric integration of psychotherapy

Across the Atlantic, in the USA, the Accreditation Council for Graduate Medical Education (ACGME) and the American Board of Medical Specialties (ABMS) in 1999 recognized a group of six principles required for measuring the competency in medical education: patient care; medical knowledge; interpersonal and communication skills; practice-based learning and improvement; professionalism; systems-based practice [20, 21].

In July 2002, the Psychiatry Residency Review Committee (RRC) concluded that every residency program in psychiatry throughout the USA should implement these principles in their clinical and didactic curriculum. As a part of the process of psychiatry adapting to these principles, the Psychiatry RRC recognizes and includes competency in five different forms of psychotherapy [22–24]: psychodynamic psychotherapy; supportive psychotherapy; cognitive-behavioral psychotherapy; short psychotherapy; psychotherapy combined with psychopharmacotherapy. Implementing knowledge in these five areas creates a basis for good psychiatric education for all residents, which speaks of integration based on the current knowledge, research, and practice [10]. It is important to emphasize that tried and tested aspects of training are not discarded [25]. Training for a therapist is an evolving process that requires time, where the dialectic between personal growth and acquisition of psychotherapy skills dances with one another [17].

Collective evaluation, toward which medical residencies are aiming, is inherently unreachable for psychotherapy, and the best we can hope for is a series of formative evaluations consistent with ideas that the existence of the psychotherapist is a process rather than a final accomplishment [25]. Likewise, knowing which instruments are used to measure competency in psychotherapy is

essential [26]. It is also important to make a distinction between whether the decision of the psychiatrist is safe for practice and how competent the psychiatrist is. A recent study that examined burnout in doctors showed that there should be a change in the focus of education from the individual learning styles of doctors to the distribution of knowledge acquired through their experience in teams [27, 28]. Even if there are practical problems in measuring competency in psychotherapy, some studies showed the importance of using tests in psychodynamic psychotherapy, as well as standard candidate screening [29, 30].

Psychotherapy as a subspecialty

The question of psychotherapy as a subspecialty in psychiatry is still a discussed topic, even with the set models of education mentioned before [1, 12, 21, 16]. We believe the complex interaction, which is grounded on an important task that the psychiatrist has – to help patients in everyday practice, far outreaches the situations in the areas of psychological and social dysfunctionality. Other professionals practice psychotherapy and psychotherapeutic counseling. Several aspects should be kept in mind in order to understand the requirement for knowledge and psychotherapeutic skills in psychiatry.

First, in psychiatric practice, there are many types of therapeutic interventions, with the understanding that humans are biopsychosocial beings. Psychopharmacotherapy is included as the basis, taking into account that psychiatry itself is a medical discipline and that medicines are an expected, if not required, method of treatment. Second, psychotherapy arises from specificities of doctor-patient relationships in psychiatry, verbal and nonverbal communication, as well as emotional reactions in the diagnosis and treatment of the patient, and research which shows the possibility of aimed psychotherapy treatment due to plasticity and positive response for psychotherapeutic interventions to certain aspects of personality [3, 31–33]. Finally, as the third component, there are sociotherapy treatments, based on the understanding that recovery of an individual and group becomes whole only after there is an integration of social environments with the biological and psychological treatment, helping in better, more comprehensive understanding of development and functions of the human being [34].

Furthermore, psychiatry offers services in different organizational forms, hospital treatment, day hospitals, outpatient units, and private practice. On all levels, psychotherapy has its role, which differs from psychotherapeutic modalities to types of intervention, as the evidence shows [35, 36]. For a long time, there have been results that show that combined pharmacotherapy and psychotherapy treatments give better results than just using pharmacotherapy in various clinical entities and age groups [33, 37]. Such is the case of treating schizophrenia, as behavioral therapy is used for resocialization and better integration [38], and psychodynamic psychotherapy helps the patient put his personal experience in words, helping them, this way, to reduce symptoms and allowing them to continue work, education, and life [39]. There is no doubt that psychotherapy is vital in comorbid states of personality disorders and recurrent depressions [40], bearing in mind the complexity of this relationship and their combined influence, as Gajić and Pejović [41] previously wrote in 2001. Finally, there is an evident need for psychotherapy in treating personality disorders, where this type of treatment is more impactful than pharmacotherapy. In this field, there is a need for further research and also training for psychiatrists [42].

In our opinion, the psychiatrist who practices psychotherapy must also be a pharmacotherapist since they deal with compatible methods of treatment with synergistic effects [3, 31]. Studies in genetics, molecular biology, and neuroimaging formed a basis for a better insight into dynamic psychotherapy through understanding that early emotional experience, trauma, and intensive interactions between patient and therapist have an influence on gene expression, synaptic neuroplasticity, and metabolism of the brain in certain regions [3]. Furthermore, we have to bear in mind that administering medication, besides its main role, has a phantasmal, irrational, and symbolic role. Based on that, a trained therapist can understand situations in which some side-effects are not from pharmacological causes [3, 31]. This position, and the integration of psychiatrist and psychotherapist, describes the clinician of the future – someone whose treatment represents recognizing the patient as an active participant in the planning and implementation of treatment. This position has yet to become a trend as we aim to improve the quality of mental health care [43].

All this opens the question of whether, even with the described and well-established models of integration of psychotherapy in psychiatric training, a subspecialty should be even discussed [44, 45]. Josef Gregory and David Mintz from the USA and Jessica Yakeley from Tavistock Clinic in London asked the question of subspecialty in psychotherapy, relying on the current state of psychotherapy in psychiatry and the existence of Medical Psychotherapy in the UK [46]. Psychotherapy is, according to these authors, undoubtedly effective in psychiatric practice [45], its role is crucial in treating many diseases combined with pharmacotherapy, and according to meta-analysis studies, the combination is more successful than pharmacotherapy alone [47]. However, the proportion of time given to the psychiatrist for psychotherapy is decreasing [47], and the identity of psychotherapy in psychiatry is diminishing [45]. As medical insurance does not consider psychotherapy as a service of the psychiatrist, the public opinion of psychotherapy itself has changed [45].

Besides that, the ever-more present dichotomous approach decreases the importance of interpersonal aspects of psychiatric care. Giving primacy to pharmacotherapy due to the pressure from the pharmaceutical industry marketing, as well as the fact that the National Institute for Mental Health finances biological mechanisms-based research and psychopharmacology for “brain diseases”, shows us the direction of movement [48]. Meanwhile, psychiatrists are encouraged to perform a highly specialized job, while the

psychotherapeutic job, which they call “counseling”, is done by less specialized and less accountable team members [45]. From this, we can see how psychotherapy is moved to the sidelines.

All this leads to the conclusion that, at this moment, there are many contradictions, from the role of psychotherapy in psychiatry and results from research on the one side to the fact related to the implementation of that work in practice and financing on the other side. All this requires a solution. Gregory et al. [45] believe that with the development of the subspecialized discipline of psychotherapy in psychiatry, an educational structure and adequate training of the psychiatrist could be established, thus improving the status of psychotherapy, allowing for the advocacy and maintenance of psychotherapy as one of the basic skills of the psychiatrist.

The subspecialty of psychotherapy was established at the Faculty of Medicine of Belgrade University in 1978; it was a pioneering undertaking of an academically oriented and organized education. According to the documents and archive materials from one of the founders of this subspecialty, Professor Dr. Miroslav Antonijević, continuing his activity in the domain of psychotherapy, created a background for the development of an institutional approach to psychotherapy education [1]. A very important aspect of organizing that education was that it was a result of interdisciplinary cooperation between several colleges – the Faculty of Medicine, the Faculty of Philosophy, and the Faculty for Special Education and Rehabilitation. Alongside them, several health institutions participated, such as the Institute for Mental Health, Clinic for Psychiatry University Clinical Center of Serbia, and Clinic “Dr. Dragiša Mišović” [1]. The Rectorate of Belgrade University played the final role. The subspecialty of psychotherapy should be something to be proud of since it is one of a kind globally in several aspects. First of all, it was academic cooperation because the work of neuropsychiatrists and psychologists went hand-in-hand. Secondly, Faculty of Medicine of Belgrade University, way before others, established psychotherapy as a highly specialized subspecialty, including it in the program of training.

The professionalism and the need for psychotherapists at that time led to the meeting of the Association of Psychotherapists of Yugoslavia in Zagreb on September 15 and 16, 1984, where the main topic was the request of the Board of the Association of Physicians Societies of Yugoslavia to create a plan and program for specialization and subspecialization in psychotherapy. The commission of educators stated that due to the need for prevention, diagnosis, and treatment of mental disorders, and psychosomatic diseases, there was a need for additional theoretical knowledge and practical training. In this way, psychotherapy as a subspecialized (directed) discipline became a part of the field of clinical psychiatry. One prominent function of psychotherapy was also highlighted – its role in preventing mental health problems and disorders and in the social context. The commission of educators believed that psychotherapists with knowledge and practical experience could aid in the creation of healthier interpersonal relationships [1].

From the organizational view, a very important body should be highlighted – the Collegium of Supervisors. They had their own rules of procedure, accredited by the Faculty of Medicine of the University of Belgrade as the main carrier and sponsor of the subspecialization [49]. On the initiative of the Collegium of Supervisors, the Society of Psychoanalytic Psychotherapists of Serbia was created to strengthen the identity of psychoanalytic psychotherapists in Serbia. It was developed on June 22, 1991, in Belgrade, with the headquarters at the Institute for Mental Health [50]. It was developed as the first of its kind in this part of the world. It was not developed as a place of education but rather as a place where the practitioners of psychotherapy would further strengthen the identity of their field of study. The development of the subspecialty through this program of theoretical and practical training gradually becomes wholly psychoanalytical. After some colleagues and Professor Vojin Matić became accredited by the International Psychoanalytic Association, Professor Dr. Ljubomir Erić included this group of people as a part of the supervisors and training analysts for the 1998 generation. That moment closed the circle of the whole process and set it following international standards.

After more than thirty years, many psychiatrists, neuropsychiatrists, and clinical psychologists were trained. According to our records, the total number of those attending the program is 160, and the number of those who completed a subspecialty is 30 in this period of thirty years. What is keeping that number from increasing? There is a lot to analyze from this, and it would be a topic for another paper. Some of these have been answered in our previous studies [1]. The most mentioned reason is rigorous training, following international standards for the area of psychoanalytic and psychodynamic psychotherapy. The training program and type of schooling required by the Faculty of Medicine at the University of Belgrade are also mentioned. It requires the following from the most recent generations: 300 hrs of individual psychotherapy, 150 hrs of individual supervision, and 100 hrs of group supervision. The academic program of the Faculty of Medicine includes passing general exams (research methodology, statistics), colloquiums in theory and practice of psychotherapy, and the final exam in front of a commission of university professors, including research, writing, and defence of the subspecialty thesis paper. Different aspects of training, experience in this training, and the demands it places in front of candidates have already been discussed in our previous paper [51].

Psychiatrists, as well as other colleagues in healthcare systems, psychologists, and specialists in medical psychology, work with patients, not clients, or in the words of founders of psychotherapy as a profession from the University Sigmund Freud in Vienna, affected persons. It is of great importance to know that at the University of Sigmund Freud in Vienna, treatment of affected persons is conducted at the Psychotherapy Outpatients Clinic, a teaching institution of this university [52]. Why is this the case? Of course, they believe that psychotherapeutic training, even

independent of psychiatry and psychology, is academic; a treatment that their students administer in the process of education and training must be administered through institutions where that education and training take place.

Conclusion

The role of psychotherapy in clinical psychiatry still exists as a very contemporary topic. The history of psychoanalysis shows its strong influence on twentieth-century clinical psychiatry. Aside from the revolution of biological psychiatry, with new medications which brought progress in the treatment of psychiatric patients, the end of this trend brought the realization that future psychiatrists in serious educational systems must have a base of knowledge in psychotherapy. With our experience in the UK, where medical psychotherapy developed into a fellowship, we believe it speaks enough about the importance of this topic.

Psychiatrists – medically trained professionals – have to face challenges every day in their practice, which requires basic psychotherapeutic knowledge, and also subspecialist knowledge. The complexity of conditions in mental health is extremely significant, such that it should not be left without the control of several different professionals trained through informal systems, who are often incapable of recognizing the dangers of working with high-risk groups of patients. It is also important to consider that psychiatrists themselves in the frame of psychotherapeutic training have to learn from specialists in psychiatry and medical psychology on the academic level through nationally accredited programs. On the whole, in this paper, we gave an example of the integration of psychotherapy in psychotherapeutic training and subspecialty training in Serbia and the models for this integration, such as those in the UK and those being considered in the USA.

As it comes to psychiatrists, the model that should continue in Serbia must be both integration and subspecialization. Subspecialization should be modernized with modalities in a similar or even the same fashion as the European and American models. This topic is extremely serious and so important to be left without regulation.

R E F E R E N C E S

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Common surgical practice in the treatment of patients with popliteal artery aneurysm among vascular centers in Serbia

Savremeno lečenje bolesnika sa aneurizmom poplitealne arterije u vaskularnim centrima u Srbiji

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Abstract

Background/Aim. Popliteal artery aneurysm (PAA) is the most common peripheral artery aneurysm and the second most common aneurysm following abdominal aortic aneurysm (AAA). Still, its incidence is rare, and treatment is non-standardized. The collection of data in a multicenter registry could improve the diagnosis and treatment of PAA. SerbVasc is a newly established data collection collaboration among vascular centers in Serbia. The aim of this study was to present common surgical practices in the diagnosis and treatment of patients with PAA in hospitals in Serbia. **Methods.** Vascular centers in Serbia that accepted the invitation collected data retrospectively concerning patients operated on for PAA from 2012 to 2018. Data regarding symptoms, preoperative diagnostics, vascular and endovascular techniques, and postoperative results were collected. This data set was submitted to the VASCUNET international project of PAA for data analysis between the countries. The same data set was used for a detailed analysis of the contemporary treatment of PAA in six hospitals in Serbia: University Clinical Center of Serbia, "Dedinje" Cardiovascular Institute, Military Medical Academy, University Clinical Center Novi Sad, University Clinical Center Niš, and General Hospital Užice. **Results.** From 2012 to 2018, in six hospitals in Serbia, data for 342 procedures on treating PAA were collected for 329 (96.2%) men and only 13 (3.8%) women. The incidence of PAA repair was 6.8 operations *per* million inhabitants a year. The mean age of patients was 64.34 years (ranging from 29 to 87). A total of 223 (65.8%) elective procedures were performed. Amputation and hospital survival were considered the main outcomes. Thrombosis was recorded in 110 (32.5%) patients as a cause for surgery, and rupture was recorded in 5 patients. The mean diameter of the aneurysm was 35.3 mm, and a slightly larger diameter was recorded in ruptured aneurysms – 43.8 mm on average. Both synthetic and vein grafts were used in elective and urgent procedures equally. Endovascular procedures were performed in 6 (1.8%) cases. **Conclusion.** This study confirms the importance of registry-based collection of data and their analysis. It showed that the national incidence of PAA in Serbia is low and that well-organized, even institution-based, screening algorithms should improve identifying such patients and increase the number of electively treated PAA. Educating vascular surgeons to use the posterior approach could improve vascular healthcare.

Key words:
endovascular procedures; popliteal artery aneurysm; serbia; vascular surgical procedures.

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Apstrakt

Uvod/Cilj. Aneurizma poplitealne arterije (APA) je najčešća aneurizma perifernih arterija i druga po učestalosti posle aneurizme abdominalne aorte (AAA). Međutim, njena učestalost je mala, a lečenje nestandardizovano. Formiranje multicentričnog nacionalnog registra moglo bi unaprediti dijagnostiku i lečenje APA. SerbVasc je novoformirani registar podataka o operisanim bolesnicima u vaskularnim centrima Srbije. Cilj rada bio je da se prikažu podaci iz hirurške prakse o dijagnostici i lečenju bolesnika sa APA u bolnicama u Srbiji. **Metode.** Vaskularni centri u Srbiji koji su prihvatili učešće u istraživanju prikupili su, retrospektivno, podatke o bolesnicima operisanim zbog APA u periodu od 2012. do 2018. godine. Prikupljeni su podaci o simptomatologiji, preoperativnoj dijagnostici, otvorenim i endovaskularnim tehnikama lečenja, kao i o rezultatu postoperativnog lečenja. Prikupljeni podaci su, radi analize između zemalja učesnica, prosleđeni VASCUNET internacionalnom projektu za APA. Isti podaci korišćeni su u analizi savremenog hirurškog lečenja APA u šest bolnica u Srbiji: Univerzitetski klinički centar Srbije, Institut za kardiovaskularne bolesti „Dedinje", Vojnomedicinska akademija, Univerzitetski klinički centar Novi Sad, Univerzitetski klinički centar Niš i Opšta bolnica Užice. **Rezultati.** U periodu od 2012. do 2018, u šest bolnica u Srbiji prikupljeni su podaci o 342 procedure lečenja APA, kod 329 (96,2%) muškaraca i kod samo 13 (3,8%) žena. Učestalost hirurškog lečenja APA iznosila je 6,8 intervencija na million stanovnika godišnje. Prosečna starost bolesnika iznosila je 64,34 godine (od 29 do 87 godina). Izvršeno je ukupno 223 (65,8%) elektivnih intervencija. Amputacija i bolničko preživljavanje su smatrani glavnim ishodima lečenja. Tromboza, kao uzrok operacije, je zabeležena kod 110 (32,5%) bolesnika, a ruptura kod pet. Prosečan dijametar aneurizme iznosio je 35,3 mm, a nešto veći dijametar zabeležen je kod rupturiranih aneurizmi – prosečno 43,8 mm. Sintetski i venski graftovi su jednako korišćeni, kako u elektivnim, tako i u hitnim operacijama. Endovaskularne procedure korišćene su kod 6 (1,8%) bolesnika. **Zaključak.** Sprovedeno istraživanje potvrđuje vrednost postojanja registra podataka i njihove analize. Pokazalo se da je učestalost APA u Srbiji niska i da dobro organizovani *skrining*, čak i u okviru pojedinačnih bolnica, može značajno unaprediti prepoznavanje bolesnika sa APA i povećati broj elektivno operisanih bolesnika. Edukacija vaskularnih hirurga o upotrebi posteriornog pristupa može unaprediti zdravstveni sistem.

Ključne reči:
endovaskularne procedure; a. poplitea, aneurizma; srbija; hirurgija, vaskularna, procedure.

Introduction

Popliteal artery aneurysm (PAA) is the most common peripheral artery aneurysm and the second most common aneurysm besides abdominal aortic aneurysm (AAA) [1]. The incidence of PAA is 7/100,000 in men and 1/100,000 in women. In 50% of cases, it is present bilaterally, and in 30–50%, it is accompanied by AAA [1–4]. Complications of PAA include thrombosis, embolic events, rupture, and compression of adjacent structures. High morbidity and amputation rate caused by PAA can be prevented by surgical treatment, which is advised for all symptomatic PAA and asymptomatic PAA larger than 2 cm [2–7]. Treatment modalities are heterogeneous, and they are mostly diverging between vascular and endovascular solutions. Open surgical treatment differs among centers and countries in terms of approach to PAA and selection of conduit [8–11]. Endovascular techniques used are exclusion with a covered stent, catheter-guided thrombolysis, and hybrid procedures [12–15]. Due to the variety of morphological features of PAA and diverse patient conditions, from young and healthy to old and fragile, there is no consensus for optimal treatment tactics and modality, while the low prevalence of this disease makes experienced data collection of the results rather difficult. Registry-based data may be used to collect experience, analyze common practice and assess the incidence and diagnostic modalities. A recent publication reported data collected in VASCUNET collaborative group showing varieties between countries. VASCUNET is a committee that works under the auspices of the European Society for Vascular Surgery and presents a collaboration of registries of vascular surgery in Europe, Australia, New Zealand, and Brazil. That is the first publication of the VASCUNET group with the Serbian dataset included [2].

The aim of this study was to present common surgical practices in the diagnosis and treatment of patients with PAA in hospitals in Serbia.

Methods

In order to participate in the VASCUNET report of 10,764 PAA cases published by Grip et al. [2] in 2020, main vascular centers in Serbia collected data retrospectively on patients operated on due to PAA from 2012 to 2018. All centers were invited, and those who accepted the invitation contributed to the study. This data set was used for detailed analysis of the contemporary treatment of PAA in six hospitals in Serbia: University Clinical Center of Serbia (UCCS), "Dedinje" Cardiovascular Institute (DCI), Military Medical Academy (MMA), University Clinical Center Novi Sad (UCCNS), University Clinical Center Niš (UCCN), and General Hospital Užice (GHU). All variables included in the dataset were determined by the VASCUNET group and were previously agreed upon between 14 countries participating in the Delphi consensus processes on chronic lower limb ischemia [16], acute limb ischemia [17], and previous VASCUNET reports on PAA [18].

Variables collected were the following: country, hospital, patient age, patient ID, gender, admission date, admission mode (indications for operative treatment were defined as elective and emergency due to thrombosis, ischemia, or rupture of PAA), diabetes mellitus, cardiac history (ischemic heart disease or congestive heart disease), current smoker

status, pulmonary history, cerebrovascular event history (history of a transient ischemic attack or stroke), hypertension history, indication, side of operation, ankle-brachial index (ABI), the diameter of the aneurysm, thrombosis, run-off (one, two, or three vessels), operation date, procedure (open, endovascular, hybrid), access route (medial, posterior, other, endovascular), proximal anastomosis site (iliac, femoral, popliteal), distal anastomosis site (popliteal above the knee, popliteal below the knee, crural), graft type (vein, synthetic, composite), additional open procedure, additional endovascular procedure, preoperative thrombolysis, perioperative thrombolysis, discharge date, wound complication, hemorrhage, fasciotomy, graft patent at discharge, graft patent at 30 days, amputation, acute kidney injury postoperatively, acute coronary event, major stroke, renal replacement therapy, died within 30 days of surgery, date of death, symptoms, amputation, graft patent at one year, symptoms remaining after one year.

All vascular clinics based at four national university clinical centers were invited, as well as all vascular departments in general hospitals. Data from the clinics that accepted to participate and those that sent the database by the due date were included. Data collected enabled analysis of incidence, demographics, indications, comorbidities, diameter, operative procedures and strategies, postoperative complications, and follow-up characteristics. Since this was a retrospective collection of data, some of the data were missing in every center. UCCS did not report symptoms at follow-up, MMA and UCCN did not report postoperative ABI, and no center reported previous cerebrovascular events. All postoperative data for UCCS and UCCN were collected on discharge. UCCNS used 30-day data for all the records, and other hospitals used both. Postoperative data were obtained on discharge in 242 cases, and in 95 cases, postoperative data were obtained at 30 days. Data for five cases were missing.

A population of 7.11 million was used for analysis, and it was calculated as the mean of the population in the first and last year of the time period.

The SPSS software package version 20.0 (IBM, Armonk, New York, USA) was used for statistical analysis. Statistical comparisons were performed with cross tabulation and comparison of mean values.

Results

From 2012 to 2018, a total of 342 procedures due to PAA were collected in six hospitals in Serbia. The incidence of PAA repair was 6.8 operations *per* million inhabitants a year. The mean age of patients during the period was 64.34 years, ranging from 29 to 87 years. Operated patients were predominantly men, 329 (96.2%); only 13 (3.8%) were women.

The overall incidence from 2012 to 2018 was 6.8 operations *per* million inhabitants a year. Incidence for the Novi Sad region was 28.1 operations *per* million inhabitants a year, Niš 6.6 operations *per* million inhabitants a year, and Užice 29.3 operations *per* million inhabitants a year.

Preoperative cardiac disease and hypertension history, including smoking habits, differed between the hospitals. Incidences of diabetes mellitus, cardiac, pulmonary, hypertension history, and smoking were analyzed for 339 procedures; data for three procedures were missing. Cerebrovascular disease history data was not provided. Data are presented in Table 1.

A total of 223 (65.8%) elective procedures were performed. Out of 115 (34%) emergency procedures, the indication for the treatment was thrombosis in 110 (32.5%) and rupture in 5 (1.5%) cases. Procedures for left-sided PAA were performed in 226 (66.1%) and for right-sided in 116 (33.9%) cases. Imaging methods were used to record aneurysm size and run-off vessels. The mean diameter of the aneurysm was 35.3 mm, ranging from 13 mm to 90 mm. For ruptured aneurysms, the diameter is larger, 43.8 mm on average, ranging from 28 to 60 mm. Run-off vessel data, defined as one, two, or three vessels present, were eligible for 159 (46.5%) patients.

More than half of all the procedures, 177 (51.7%), were performed in UCCS, and the other half was divided among the other hospitals. The majority of emergency patients were treated in the same hospital – 78/177 (44.1%), mostly due to thrombosis. Out of 336 open procedures, the medial approach was used in 237 (69.3%), the posterior approach in 98 (28.7%), and the extended medial in 1 (0.3%). The proximal anastomotic site was iliac, femoral, and popliteal artery in 1 (0.3%), 140 (42%), and 192 (57.7%) procedures, respectively, while for nine proce-

Table 1

Distribution of patients by sex, age, and preoperative history among hospitals in Serbia

Variable	Total	UCCS	DCI	MMA	UCCNS	UCCN	GHU	*p*-value
Male	329 (96.2)	173(97.7)	37 (90.2)	28 (96.5)	64 (95.5)	12(100)	15 (93.7)	0.311
Female	13 (3.8)	4 (2.3)	4 (8.8)	1 (3.5)	3 (4.5)	0 (0)	1 (6.3)	0.311
Age	64.34	64.12	63.20	64.59	66.06	59.92	65.38	0.344
Diabetes	55 (16.2)	34 (19.2)	6 (15.8)	3 (10.3)	6 (8.95)	2 (20)	4 (33.3)	0.367
Cardiac history	90 (26.6)	37 (20.9)	14 (36.84)	6 (20.7)	22 (32.8)	1 (9.1)	10 (62.5)	**0.002**
Pulmonary history	42 (12.4)	17 (9.6)	5 (13.2)	2 (6.9)	13 (19.4)	1 (8.3)	4 (25)	0.176
Hypertension	264 (77.9)	129(72.9)	38 (100)	22 (75.9)	51 (76.1)	10(83.3)	14 (87.5)	**0.012**
Current smoker	117 (34.5)	63 (35.6)	11 (28.9)	5 (17.2)	19 (28.4)	7 (58.3)	12 (75)	**0.001**

UCCS – University Clinical Center of Serbia; DCI – Cardiovascular Institute "Dedinje"; MMA – Military Medical Academy; UCCNS – University Clinical Center Novi Sad; UCCN – University Clinical Center Niš; GHU – General Hospital Užice.

All values are expressed as numbers (percentages). Bolded values are statistically significant.

dures, there were no data on the proximal anastomotic location. The distal anastomosis was performed above the knee in the popliteal artery, below the knee in the popliteal artery, and in the crural arteries in 48 (14.4%), 260 (78.8%), and 25 (7.5%) procedures, respectively. The synthetic graft was used in 172 (51.8%), the vein graft in 154 (46.4%), and the composite graft in 6 (1.8%) procedures, while there were no data for ten procedures. Both synthetic and vein grafts were used in elective and urgent procedures equally. In emergency cases, the synthetic graft was used more often than the vein graft for thrombosis and rupture in all cases eligible. Additional open or endo procedures were needed in 14.1%. Thrombolysis was performed preoperatively in 1 and perioperative in 11 cases. Endovascular procedures were performed only in 6 (1.8%) cases, all for elective admissions in DCI. The distribution of the number of procedures in six centers, as well as other preoperative and intraoperative parameters, is presented in Table 2.

Surgical wound complications demanding surgical treatment were present in less than 1% – 23 patients, hemorrhage occurred in 9 (2.7%) cases, and fasciotomy was indicated in 14 (4%) patients; all of this was more frequent in emergency cases. Amputation was performed in 10 (2.8%) patients, 5 (2.2%) in elective and 5 (4.7%) in urgent patients, 3 (2.7%) in thrombosed, and 2 (1.7%) in ruptured PAA. No major strokes were recorded, and 4 (1.2%) patients had an acute coronary event (acute coronary syndrome, myocardial infarction, serious arrhythmia, cardiac failure). One patient (0.3%) had a renal impairment that needed renal replacement therapy. Four patients (1.2%) died at 30 days: two in elective and two in the emergency group. Moreover, there were 11 (3.2%) graft occlusions recorded. Data for amputation were missing in 25 cases and also for graft patency at 30 days in 37 patients. Follow-up at one year recorded amputation, graft patency at one year, and persistence of the symptoms. Amputation rate data were eligible for 272 cases, graft patency

Table 2

Indications, preoperative imaging, and type of surgical procedure

Variable	Total	UCCS	DCI	MMA	UCCNS	UCCN	GHU
Number of procedures	342 (100)	177 (51.77)	41 (12.0)	29 (8.5)	67 (19.6)	12 (3.5)	16 (4.7)
Indication							
elective	223(65.8)	98 (55.4)	35 (92.1)	22 (75.9)	46 (68.7)	10 (83.3)	12 (75)
thrombosis	110 (32.5)	78 (44.1)	3 (7.9)	5 (17.2)	19 (28.4)	1 (8.3)	4 (25)
rupture	5 (1.5)	0	0	2 (6.9)	2 (2.9)	1 (8.33)	0
diameter	35.4	33.5	33.9	37.6	39.3	35.3	35.3
right	116 (33.9)	31 (17.5)	22 (53.6)	12 (41.4)	35 (52.2)	6 (50)	10 (62.5)
left	226 (66.1)	146 (82.5)	19 (46.3)	17 (58.6)	32 (47.8)	6 (50)	6 (37.5)
Run-off							
one vessel	43(26.9)	5 (13.9)	5 (13.9)	15 (51.7)	11 (16.4)	10 (83.3)	2 (12.5)
two vessels	46 (28.8)	7 (19.4)	7 (19.4)	6 (20.7)	26 (38.8)	1 (8.3)	6 (37.5)
three vessels	70 (43.8)	23 (63.9)	23 (63.9)	8 (27.6)	30 (44.8)	1 (8.3)	8 (50)
Procedure							
open	336 (98.3)	177 (100)	35 (85.4)	29 (100)	67 (100)	12 (100)	16 (100)
endovascular	6 (1.8)	0	6 (14.6)	0	0	0	0
Access route							
medial	237 (69.3)	88 (49.7)	35 (85.4)	26 (89.7)	65 (97)	11 (91.7)	11 (68.8)
posterior	98 (28.7)	89 (49.3)	0	3 (10.4)	2 (3)	0	5 (31.3)
other	1 (0.3)	0	0	0	0	1 (8.3)	0
endovascular	6 (1.8)	0	6 (14.6)	0	0	0	0
Proximal anastomosis							
iliac	1 (0.3)	0	0	1 (3.5)	0	0	0
femoral	140 (42)	72 (40.9)	16 (45.7)	12 (41.4)	25 (37.3)	10 (100)	5 (31.3)
popliteal	192 (57.7)	104 (59.1)	19 (54.3)	16 (55.2)	42 (62.7)	0	11 (68.8)
Distal anastomosis							
popliteal above knee	48 (14.4)	16 (9.1)	6 (17.1)	7 (24.1)	19 (28.4)	0	0
popliteal below knee	260 (78.8)	152 (86.4)	29 (82.9)	15 (51.7)	42(62.7)	8 (80)	14 (87.5)
crural	25 (7.5)	8 (4.6)	0	7 (24.1)	6 (8.9)	2 (20)	2 (12.5)
Graft type							
vein	154 (46.4)	80 (45.7)	14 (0.4)	7 (24.14)	36 (53.7)	8 (80)	9 (56.2)
synthetic	172 (51.8)	94 (53.7)	21 (0.6)	17 (58.6)	31 (46.2)	2 (20)	7 (43.7)
composite	6 (1.8)	1 (0.5)	0	5 (17.2)	0	0	0
additional open procedure	41 (12)	18 (10.2)	4 (9.8)	9 (31)	6 (8.9)	2 (16.7)	2 (12.5)
additional endo procedures	7 (2.1)	6 (3.4)	0	0	1 (1.5)	0	0
preoperative thrombolysis	1 (0.3)	0	0	0	0	0	1 (6.3)
perioperative thrombolysis	11 (3.2)	11 (6.2)	0	0	0	0	0

For abbreviations see Table 1. All results are shown as numbers (percentages) except diameter, which is shown in millimeters.

Table 3

Follow-up data

Variable	Total	UCCS	DCI	MMA	UCCNS	UCCN	GHU
Wound complication	23(0.9)	9 (5.1)	0	3 (10.3)	9 (13.4)	1 (8.3)	1 (6.3)
Hemorrhage	9 (2.7)	4 (2.3)	0	0	4 (6)	1(8.3)	0
Fasciotomy	14 (4.1)	6 (3.4)	0	1 (3.5)	3 (4.5)	1 (8.3)	3 (18.8)
Discharge patency	317 (96.3)	173 (98.3)	31 (96.9)	26 (89.7)	64 (95.5)	9 (100)	14 (87.5)
One-month patency	293 (96.1)	155 (98.7)	26 (96.3)	26 (89.7)	63 (94.0)	9 (100)	14 (87.8)
Amputation	10 (2.8)	2 (1.3)	1 (2.8)	2 (6.9)	3 (4.5)	2 (18.2)	0
Coronary event	4 (1.2)	0	0	1 (3.5)	1 (1.5)	1 (8.3)	1 (6.3)
Cerebrovascular event	0	0	0	0	0	0	0
Renal replacement therapy	1 (0.3)	0	0	1 (3.5)	0	0	0
Died in 30 days	4 (1.2)	1 (0.6)	0	0	0	1 (8.3)	2 (12.5)
Occlusion at 30 days	11 (100)	2 (18.2)	1 (9.1)	3 (27.3)	4 (36.3)	0 (0)	1 (9.1)
Symptoms remaining at 30 days							
no symptoms	305 (96.2)	157 (99.4)	32 (88.9)	28 (96.6)	67 (100)	8 (66.7)	13 (86.7)
claudication	11 (3.5)	1 (0.6)	4 (11.1)	1 (3.5)	0	4 (33.3)	1 (6.7)
gangrene	1 (0.3)	0	0	0	0	0	1 (6.7)
One year follow-up							
amputation in one year	15 (5.5)	2 (1.6)	3 (8.3)	0	7 (12.3)	2 (16.7)	1 (6.7)
one year patency	247 (91.8%)	119 (97.6%)	33 (91.7)	25 (89.3)	46 (82.1)	10 (100)	14 (93.3)
symptoms at one year	20 (15.3)		9 (27.3)	1 (3.6)	9 (16.1)	0	1 (6.7)

For abbreviations see Table 1. All values are expressed as numbers (percentages).

for 269, and persistence of the symptoms for 132 cases. Postoperative complications and follow-up are presented in Table 3.

Discussion

To participate in the VASCUNET report on PAA, the Serbian vascular service joined the database reports of PAA for seven years. Six hospitals in Serbia participated, and 342 cases were collected. Analysis among vascular centers in Serbia and departments has been performed assessing incidence, demographics, indications, comorbidities, diameters, operative procedures and strategies, postoperative complications, and follow-up characteristics of common surgical practice for PAA treatment.

For centuries surgeons have been dealing with PAA. From the Antyllus proximal and distal ligation in the 3rd century AD, Hunters proximal ligation in 1785, Matas endoaneurysmorrhaphy to revascularization after exclusion of aneurysm with the popliteal vein (Goyanes,1905), end to end anastomosis (Enderlen, 1907), saphenous vein (Pringle, 1913), and synthetic graft (Crawford, 1957), surgical treatment has evolved [19, 20]. Accessibility of modern diagnostic tools, especially Doppler ultrasound and multidetector computed tomography present in every major hospital, makes the detection of PAA easy. Extension of the disease, inflow and run-off vessels, and the quality of the great saphenous vein as a conduit can be easily obtained, and operative tactics can be managed accordingly [21, 22]. However, the incidence of PAA detection is not as expected, and the results of surgical treatment are still troublesome.

There were 342 procedures for PAA for seven years in six vascular departments. The overall incidence from 2012 to 2018 is 6.8 operations *per* million inhabitants a year. That is far behind the incidence reported in Sweden, where a screening program for AAA exists and considers routine examination of popliteal arteries [2]. Only Malta, New Zealand, and Iceland have a lower incidence of PAA operation *per* million inhabitants. Information about the number of amputations performed due to prolonged acute ischemia would also be very informative for vascular healthcare in Serbia. Only the Užice County could be calculated for incidence due to the existence of the same group of patients coming from the same area. Užice has 28 cases of PAA *per* million inhabitants a year which is far more than the country's average. Low incidence is either a manifestation of good primary prevention or a low incidence of early primary detected PAA.

Consequently, 115 (34%) procedures were established overall for emergency cases. Thrombosis was indicated in 32.5% of all cases, and 5 (1.5%) patients had a rupture. UCCS had the highest number of operations for thrombosis, 44.1% of all cases, and DCI had the lowest number of operations, less than 8%. That comes as a consequence of the organization of the national healthcare system, where UCCS is open 24/7 for vascular emergencies. A more profound analysis of health care service is necessary to define whether such a concept is beneficial to the patients. Women had smaller aneurysm diameters than the average of 30.45 mm and fewer (15%) urgent operations, all for thrombosis. Emergency-treated ruptured aneurysms, all recorded in men, were larger than the average (43.8 mm). The diameter of ruptured PAA ranged from 28 to 59 mm. All ruptured PAA were operated on using a medial approach.

The medial approach was used in 237 (69.3%) cases and posterior in 98 (28.7%) cases. Surgeons in UCCS used both methods evenly, while the other five hospitals relied on medial exposure predominantly. All centers used both vein and synthetic grafts for arterial reconstruction. The composite graft was used in six cases – five in MMA and one in UCCS. Each approach has advantages and limitations; how-

ever, both provide options for every particular patient. Organized institutional screening might increase the number of diagnosed patients and increase the volume of procedures in all centers, which might contribute to education and training in the dorsal approach.

Endovascular treatment was used in 1.8% of cases compared to New Zealand's 58% [2]. Vascular surgeons in Serbia used the endovascular technique the least compared to other countries [2]. There are obvious reasons like financing, accessibility, experience in stenting, as well as the mindset of surgeons in Serbia. On the other side, endovascular treatment of PAA has not been proven to be superior to open repair. The high incidence of complications prevents surgeons in Serbia from spending limited resources on such a procedure. A stepwise increase in the number of these procedures in highly selected patients may be accepted. Considering the age and comorbidities of patients presented, endovascular treatment should be used more routinely in common surgical practice.

Our study has some limitations. Not all hospitals with vascular surgery services participated. Still, these are mostly low-volume hospitals with very few potential cases of PAA, and most of the patients from these hospitals were already referred to those hospitals that were a part of this study. This study is a retrospective collection of data collected prospectively, which explains some of the missing data in our database. Data needed for such analysis of diagnostic methods, surgical techniques, and basic outcomes were not missing. This study was unable to address the number of primary amputations performed due to thrombosed PAA and prolonged leg ischemia. Considering the low incidence of PAA compared to other countries, such a number might be substantial and should be evaluated.

We were unable to include in the paper the severity of limb ischemia (acute, subacute, and chronic) due to thrombosed PAA, as this information is missing in the data set. As this is our first report of Serbian VASCUNET data, we will be stricter in quality control of included data in the data set.

Conclusion

This study confirms the importance of registry-based collection of data and their analysis. It showed that the national incidence of PAA in Serbia is low and that well-organized, even institution-based, screening algorithms should improve such findings and increase the number of electively treated patients. Education of vascular surgeons to use the posterior approach could improve vascular healthcare.

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Perinatal hypoxia as a risk factor for a more severe lexical-semantic deficit in children with developmental language disorder

Perinatalna hipoksija kao faktor rizika od težeg leksičko-semantičkog deficita kod dece sa razvojnim jezičkim poremećajem

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Abstract

Background/Aim. There is a small body of literature on the influence of perinatal hypoxia (PH) on language outcomes at a later age. Correspondingly, there are no studies on the influence of PH on the extent and severity of language deficits in children with developmental language disorder (DLD). The aim of this study was to examine the differences in lexical-semantic (LS) abilities in DLD children with the presence of PH (DLDph) and DLD children without any neurological risk factors (DLDwnrf). **Methods**. The study sample consisted of 96 children aged 5 to 8 years, divided into three groups: 25 children in the DLDph group, 30 children in the DLDwnrf group, and 41 typically developing (TD) peers. To compare age-related differences, an additional categorical variable was formed with two age groups – preschool and school-age children (5–6 and 7–8 years, respectively). LS abilities were investigated with specific measures for assessing the expressive vocabulary (EV) size, semantic processing (SP) skills, and lexical productivity (LPr). To assess LPr, measure for calculating lexical diversity from speech sample was applied. **Results**. Significant differences were observed between DLDph and DLDwnrf children on the SP assessment ($p < 0.05$) but not on the EV ($p = 0.350$) and LPr ($p = 0.118$) assessment. However, a detailed analysis of developmental tendencies between preschool and early school-age children showed that DLDph children progressed significantly only in the domain of EV ($p < 0.01$), while DLDwnrf children progressed significantly in the domain of EV and SP skils ($p < 0.001$). Regarding LPr developmental tendencies, no significant progress was observed in either of the DLD groups. **Conclusion**. In DLDph children, a more severe extent of LS deficit in the area of SP abilities can be related to PH. Similarly, PH can contribute to slower progress in a wider spectrum of LS abilities.

Key words:
brain; hypoxia; language disorders; risk factors.

Apstrakt

Uvod/Cilj. U literaturi postoji mali broj radova o uticaju perinatalne hipoksije (PH) na jezičke sposobnosti dece starijeg uzrasta. Takođe, ne postoje studije o uticaju PH na obim i težinu jezičkog deficita dece sa razvojnim jezičkim poremećajem (RJP). Cilj rada bio je da se ispitaju razlike u leksičko-semantičkim (LS) sposobnostima dece sa RJP i sa istorijom PH (RJPph) i dece sa RJP bez neuroloških faktora rizika (RJPbnfr). **Metode**. Uzorak je činilo 96 dece uzrasta od 5 do 8 godina, svrstanih u tri grupe: RJPph grupa (25 dece), RJPbnfr grupa (30 dece) i grupa od 41 tipično razvijene (TR) dece istog uzrasta. U cilju poređenja razlika koje zavise od uzrasta, formirane su i dve dodatne starosne grupe – deca predškolskog i deca školskog uzrasta (5–6 i 7–8 godina, redom). Za merenje LS sposobnosti primenjeni su specifični testovi za procenu obima ekspresivnog vokabulara (EV), semantičkog procesiranja (SP) i leksičke produktivnosti (LPr). Za procenu LPr primenjena je mera računanja leksičke raznovrsnosti u uzorku spontanog govora. **Rezultati**. Rezultati su pokazali statistički značajne razlike između grupa RJPph i RJPbnfr na testu procene SP ($p < 0,05$), ali ne i na testovima za procenu EV ($p = 0,350$) i LPr ($p = 0,118$). Međutim, detaljna analiza razvojnih tendencija dece predškolskog i ranog školskog uzrasta pokazala je da su deca iz grupe RJPph značajno napredovala samo u domenu EV ($p < 0,01$), dok su deca iz grupe RJPbnfr značajno napredovala u domenu EV i SP ($p < 0,001$). Što se tiče razvojnih tendencija u domenu LPr, ni u jednoj od dve grupe sa RJP nije utvrđen značajan napredak. **Zaključak**. Kod RJPph dece, teža forma LS deficita u oblasti sposobnosti SP može biti povezana sa PH. Takođe, PH može doprineti sporijem napredovanju šireg spektra LS sposobnosti.

Ključne reči:
mozak; hipoksija; jezički poremećaji; faktori rizika.

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Introduction

Developmental language disorder

According to the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria, developmental language disorder (DLD) is a neurodevelopmental disorder characterized by difficulties in vocabulary, syntactic abilities, and discourse skills, which can be manifested in expressive and/or receptive language and through several modalities and can significantly impair communicative, social, academic and professional functioning [1]. DLD is characterized by a delay or abnormality in expressive and/or receptive language abilities in the absence of general cognitive deficits, autism, hearing impairment, social and emotional disorders, and severe environmental deprivation [2].

There is a well-founded viewpoint that the underlying mechanism in neurodevelopmental disorders is an atypical pattern during intrauterine brain development and that relatively mild abnormalities affecting limited brain regions can lead to difficulties in developing higher cognitive functions [3]. Data from the literature indicate the presence of various lexical-semantic (LS) deficits in DLD children. In other words, these children are characterized by a significant delay in first-word acquisition [4, 5]. Some authors consider this symptom in DLD children the first key symptom of speech and language development delay [2, 3]. DLD children also have significantly underdeveloped expressive vocabulary (EV) and receptive vocabulary (RV) skills compared to their typically developing (TD) peers [6]. In accordance with poor vocabulary, these children also have word-finding difficulties [7]. Likewise, DLD children learn new words significantly slower and harder compared to TD children [8, 9]. However, word-finding difficulties in these children are not only due to retrieval difficulties but also to poor semantic representations and deficits in LS organization and processing [10–12]. In addition to the above, DLD children also have significant difficulties using words in spontaneous speech. Namely, studies of lexical diversity (LD) in speech samples of DLD children showed that these children have a significantly lower usage of all types of words [13, 14] and significantly fewer content words (nouns, verbs, adjectives) [13, 15] compared to TD peers.

Although DLD is a disorder usually diagnosed at an early preschool age with a good language outcome at a later age [1], numerous data from the literature indicate that these children can have significant difficulties at school age. Difficulties that these children have at school age are mostly manifested within LS [11, 13] and pragmatic abilities [16]. Given the importance of LS abilities for mastering academic skills, research in this population is of great importance for the academic outcomes of these children.

Perinatal hypoxia and language development

Perinatal hypoxia (PH) is a term that refers to the period before, during, and after birth in which a fetus or child is exposed to a reduced amount of oxygen in cells and tissues, which can lead to serious brain damage. The development of language and other cognitive abilities has been most studied in children who have developed hypoxic-ischemic encephalopathy (HIE) due to a severe form of PH. Data from some of these studies have shown that children with a history of HIE may have underdeveloped speech and language abilities at school age, including reading and writing difficulties, even in the absence of more severe cognitive or motor difficulties [17]. Results of some studies show that these children may have average language skills measured by general batteries of tests for cognitive abilities assessment [18]. In a recent study, Chin et al. [19] investigated the language abilities of preschool children with a history of moderate and severe HIE at birth. The authors assessed language abilities with batteries of tests for a general assessment of cognitive abilities. The results showed that children with a history of HIE could have significant difficulties with EV skills and shorter mean length of utterance (a general measure of syntactic abilities) compared to TD peers. Additionally, data from this research showed that RV skills are quite preserved in these children. However, the results of this study showed that gender and socioeconomic status are essential predictors of EV development, while the extent and severity of brain damage are more important predictors of RV in these children. According to that, the influence of HIE on the development of expressive lexical abilities (LA) is not entirely clear. On the other hand, there are no available data on the language abilities in children with a history of mild PH without sequelae in the form of HIE or some other form of brain damage. In addition, existing studies have used general assessment instruments for investigating language skills (verbal intelligence quotient – IQ, cognitive battery assessment subscales), which do not assess the structural aspects of language in detail, such as specific tests for assessing morphosyntactic, LS, and phonological or pragmatic abilities.

The only available data on the possible influence of a mild form of PH on specific language abilities comes from one larger study of LA in DLD children, showing that a group of DLD children with a presence of risk factors (RF) for slower neural maturation have poorer performance than DLD children without the presence of these factors [20]. The results of this study showed that the group of DLD children with RF for slower neural maturation had significantly worse performance in the domain of lexical processing and LD. Additionally, this group of children had slower progress within all observed LA, including naming objects and activities [20]. However, this study included children with PH and children with nonspecific encephalographic changes in the group of DLD children with RF, so the effect of PH was not investigated as an individual factor.

Present study

Several papers in the literature have studied the possible impact of PH on children's language skills. In addition, those studies used general batteries of tests for assessing language abilities, most often as part of the general cognitive abilities assessment. These types of tests are usually not sensitive to deficits that children may have within structural aspects of

language (syntactic, semantic, phonological, or pragmatic). Moreover, there are no available studies on the possible impact of PH on the severity of language deficit in DLD children, especially where PH was not severe and did not cause significant motor and cognitive disorders or where it was not considered a separate factor. Likewise, anecdotal data from practice indicate a possible severe language deficit in DLD children who suffer from PH, even in the absence of neurological or severe cognitive deficits. Accordingly, the aim of our study was a detailed examination of the possible impact of PH on the severity of lexical deficit in DLD children, using specific tests that measure three dimensions of expressive LS abilities.

Methods

Participants

The sample consisted of 96 children aged 5 to 8 years divided into three groups. Twenty-five children were diagnosed with an expressive type of DLD and a history of PH (DLDph), and 30 children were diagnosed with an expressive type of DLD without the presence of neurological risk factors (DLDwnrf) before, during, or after birth. A control sample consisted of 41 TD children without a history of DLD or other developmental disorders and without a history of neurological or sensory impairments (Table 1). All DLD children were recruited from the Institute for Psychophysiological Disorders and Speech Pathology (IPDSP) "Prof. Dr. Cvetko Brajović" in Belgrade, Serbia. All DLD children were included in speech and language therapy for 12 to 18 months. Evidence of the presence of neurological risk factors (NRF) was obtained from medical history. All DLDph children had a history of PH, 5-min Apgar score between 5 and 7, without evidence of HIE or documented neurological or motor impairment. In the first six months of the research, 21 children with DLDph who met the criteria regarding age and treatment period were included in the sample. To increase the number of school-age children, four more children were included in the sample in the next two years. A sample of DLDwnrf children who met the criteria regarding age and treatment period was formed in the first six months of the study. TD group consisted of children who were recruited from local preschools and schools, also located in Belgrade. The inclusion criterion for all groups was an IQ above 85, within the norms of average intelligence, while one child from the DLDwnrf group with IQ above average (> 109) was excluded from the sample. Data on intelligence level were taken from psychological documentation and included the general IQ and the instrument which it was assessed with. The Wechsler Intelligence Scale for Children – Revised form, which has been normed on the Serbian population, was administered for all children [21]. Only participants whose first language is Serbian were included in the sample. The research was approved by the Ethical Board of IPDSP "Prof. Dr. Cvetko Brajović" in Belgrade, Serbia (1575/19-09-2016), and for testing all children, written consent was obtained from the parents.

There were no significant differences between groups regarding age (Tables 1 and 2). Given the numerous data in

Table 1

Demographic characteristics of study participants

Variable	Groups		
	DLDph n = 25	DLDwnrf n = 30	TD n = 41
Age (months), mean ± SD	69.6 ± 9.5	73.9 ± 12.4	71.8 ± 11.6
Gender, n (%)			
girls	7 (28)	13 (43.3)	20 (48.8)
boys	18 (72)	17 (56.7)	21 (51.2)
Maternal education, n (%)			
secondary	16 (64)	13 (43.3)	22 (53.7)
tertiary	9 (36)	17 (56.7)	19 (46.3)

DLDph – developmental language disorder with perinatal hypoxia; DLDwnrf – developmental language disorder without neurological risk factors; TD – typically developing children; SD – standard deviation.

Table 2

Comparison of groups according to sociodemographic variables

Variable	Groups		Mean diff./χ^2	SE/df	*p*-value
Age	DLDph	DLDwnrf	4.227	3.070	0.391
	DLDph	TD	2.189	2.877	0.749
	DLDwnrf	TD	2.037	2.724	0.757
Gender	DLDph	DLDwnrf	0.802	1	0.370
	DLDph	TD	1.981	1	0.159
	DLDwnrf	TD	0.046	1	0.831
Maternal education	DLDph	DLDwnrf	1.581	1	0.209
	DLDph	TD	0.322	1	0.570
	DLDwnrf	TD	0.384	1	0.536

diff. – difference; χ^2 – Chi squared; SE – standard error; df – degree of freedom. For abbreviations of other terms see Table 1.

the literature that indicate the possible influence of gender [6, 22, 23] and maternal education [24–26] on children's LA, we compared groups regarding mentioned demographic variables. However, no significant differences were found between these groups of children (Table 2). To compare age differences, an additional categorical variable was formed with two age groups, preschool and school-age children (5–6 and 7–8 years, respectively). Data on the distribution of participants through age groups are given in Table 3. Comparison analysis of the participants by age groups showed that there was no statistically significant difference between all groups (DLDph vs. DLDwnrf: $\chi^2 = 0.000$, df = 1, $p = 1.000$; DLDph vs.TD: $\chi^2 = 0.000$, df = 1, $p = 1.000$; DLDwnrf vs. TD: $\chi^2 = 0.000$, df = 1, $p = 1.000$).

Instruments

To assess vocabulary size (VS), Boston Naming Test (BNT) [27] was used. The test consists of 60 black-and-white drawings of objects and assesses the ability of confrontational naming (visually evoked naming). Images of objects are sorted by usage frequency in the language, from more to less frequent concepts. The test is used to assess naming in children and adults, with and without developmental and acquired speech and language impairments. BNT is adapted for the Serbian language but is not standardized. The Serbian version of BNT has been used in several studies with Serbian-speaking children and adults with speech and language disorders [20, 28, 29]. Scores of correct answers were used for statistical analysis.

To assess LS processing skills (PS), Word Association Task (WAT) was used. Eighty words were selected from Kent and Rosanof [30] list with the addition of ten verbs in order to equalize word classes. The association test based on this list is the best studied in a linguistical manner of all available in the literature within the Birkbeck Vocabulary Project [31] in the 1980s. All words were selected to be early acquired, as highly imageable as possible depending on the word class, and high and medium frequencies according to the Children's frequency dictionary [32]. Moreover, variants of association tests are commonly used for assessing semantic processing in children with language disorders and LS organization of bilingual children [12, 33, 34]. Furthermore, the same test has been already used in a study with a larger sample of DLD children in the Serbian population [11]. Associations were coded into two categories: mature and immature associations. Mature associations are paradigmatic and syntagmatic responses, which are the indicators of a more mature and better organized semantic network resembling the one of a typical adult speaker [35]. Immature associations are phonological, unrelated, and echolalic responses, as well as omissions. These types of associations are indicators of an underdeveloped semantic network [11]. The score of mature type of associations was used for statistical analysis.

The measure of LD was used to assess lexical productivity (LPr). LD was measured by analysis of the spontaneous speech sample. A sample of spontaneous speech was obtained by retelling a story, and the fairy tale "Cinderella" was used as a stimulus task. The book "Cinderella" with pictorial material (without words) that illustrates the content was given to the children, with a request to review the picture book for as long as they needed to recall the fairy tale. After that, the book was removed, and children were asked to tell an illustrated fairy tale. It is a common method of assessing LD in people with language disorders [36, 37]. Speech samples were recorded and then transcribed according to the rules of phonological transcription of the Serbian language. From the total sample, a segment of the first 150 words was analyzed. This measure also represents the shortest speech sample of the participants. This way of segmentation has been recommended in some of the studies that have analyzed the LD of children with language disorders [38, 39]. The score of LD was calculated with the ratio of the different and total number of words in a given discourse (Type Token Ratio – TTR) [40, 41]. The lexical assessment was performed by two highly qualified speech and language therapists.

Statistical analysis

The χ^2 test was used for comparing groups of children regarding categorical variables, gender, maternal education, and age groups. Analysis of variance (ANOVA) was used for comparing groups regarding age, including differences in LA between age groups. In cases where the equivalence of variance assumption is violated, Welch's approximate method of analysis of variance was used to verify the significance of subpopulation differences in achievements in individual variables. Multiple comparisons between three groups regarding their LA were investigated with *post-hoc* analysis, Tamhane's T2 method, when the equality of variance is not assumed. Two-way ANOVA was used to investigate developmental trends in LA. SPSS software (version 26.0) was used for data analysis.

Table 3

Distribution of participants through age groups

Age (years)	Groups			Total
	DLDph	DLDwnrf	TD	
5–6	15 (60.0)	18 (60.0)	25 (61.0)	58 (60.4)
7–8	10 (40.0)	12 (40.0)	16 (39.0)	38 (39.6)
Total	25 (100.0)	30 (100.0)	41 (100.0)	96 (100.0)

Results are shown as numbers (percentages) of the participants. For abbreviations see Table 1.

Results

The results of ANOVA indicate statistically significant differences in achieving the VS, PS, and LPr tasks between DLDph, DLDwnrf, and TD children. A detailed analysis using the *post-hoc* Tamhane's T2 reveals a pattern of difference between the groups on all tasks (Table 4). Data showed that DLDph children have statistically significantly lower scores compared to DLDwnrf children on assessing PS tasks ($p < 0.05$). On the other hand, the two DLD groups do not differ significantly on VS and LPr tests, although children with DLDph have a lower average achievement (VS = 39.47 vs. 44.33; LPr = 0.29 vs. 0.34). Both DLD groups have statistically significantly lower scores compared to TD children on all tests ($p < 0.01$) (Table 4).

Further, we wanted to examine whether differences in developmental patterns between the observed groups existed. Using a two-factor ANOVA, we examined whether there are differences in developmental tendencies between preschool and school-age children in the examined groups of children. Two-way ANOVA showed specific developmental patterns in DLDph, DLDwnrf, and TD children on tasks assessing VS, PS, and LPr (Table 5).

No interaction was observed between groups of children and age on the BNT test (for VS assessment) ($F_{5; 95} = 2.565$, $p = 0.083$). All three groups of children show a similar developmental trend in vocabulary growth, with the difference in results originating from different starting points of developmental levels (Table 5).

In the case of WAT achievement (for PS assessment), a statistically significant interaction was observed between groups and age ($F_{5; 95} = 26.595$, $p \leq 0.000$) (Table 5). Group explains about 51% of results variability ($F_1 = 47.442$, $p \leq 0.000$, part $\eta 2 = 0.513$), while age explains about 20% of results variability ($F_1 = 22.898$, $p \leq 0.000$, part $\eta 2 = 0.203$). The observed pattern shows that PS improves with age but also that there are significant differences in progress between groups of children.

No interaction was observed between groups of children and age regarding LPr ($F_{5; 95} = 2.239$, $p = 0.113$). All three groups of children show a similar developmental trend regarding LPr, with the difference of starting from different developmental levels (Table 5).

However, upon observing the age differences at the subpopulation level, different developmental patterns were identified in three groups of children. Using the ANOVA test, the differences between preschool and school-age children in all three groups were compared on all three lexical tasks. Comparing the two age groups within the DLDph population, a statistically significant improvement was found only on the VS task ($F_{1; 23} = 9.884$, $p = 0.005$). On the other hand, no statistically significant differences were found between the two age groups on the PS and LPr tasks (PS – $F_{1; 23} = 1.629$, $p = 0.215$; LPr – $F_{1; 23} = 0.001$, $p = 0.980$).

Table 4

***Post-hoc* Tamhane's T2 multiple comparisons of lexical abilities between the studied groups**

Lexical abilities	Groups		Mean diff.	SE	*p*-value
Vocabulary size	DLDph	DLDwnrf	-4.867	3.2	0.350
	DLDph	TD	-28.378	2.5	**0.000**
	DLDwnrf	TD	-23.511	2.7	**0.000**
Processing skills	DLDph	DLDwnrf	-25.296	8.2	**0.010**
	DLDph	TD	-54.382	5.9	**0.000**
	DLDwnrf	TD	-29.086	6.3	**0.000**
Lexical productivity	DLDph	DLDwnrf	-0.048	0.0	0.118
	DLDph	TD	-0.205	0.2	**0.000**
	DLDwnrf	TD	-0.157	0.0	**0.000**

diff. – difference; SE – standard error. For abbreviations of other terms see Table 1. Boston naming test, mature associations, and lexical diversity were used to determine vocabulary size, processing skills, and lexical productivity, respectively.
Statistically significant values are bolded.

Table 5

Two-way ANOVA analysis of lexical abilities of the studied groups in preschool and school-age children

Lexical abilities	Age (years)	Groups			df	MS	F	*p*-value	part η^2
		DLDph	DLDwnrf	TD					
Vocabulary size	5–6	34.8 ± 8.4	37.6 ± 11.2	65.1 ± 6.1	5	196.608	2.565	0.083	0.054
	7–8	46.5 ± 10.1	54.4 ± 8	72.2 ± 9.1					
Processing skills	5–6	17.4 ± 27.5	32.0 ± 30.0	73.4 ± 11.3	5	2,094.300	26.595	**0.000**	0.596
	7–8	31.7 ± 19.0	72.9 ± 19.6	83.9 ± 10.7					
Lexical productivity	5–6	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	5	0.017	2.239	0.113	0.055
	7–8	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1					

df – degree of freedom; MS – means squares; F – statistic value for analysis of variance (ANOVA); η^2 – squared Eta.
For abbreviations of other terms see Table 1. Results are shown as mean ± standard deviation.
Boston naming test, mature associations, and lexical diversity were used to determine vocabulary size, processing skills, and lexical productivity, respectively. Statistically significant value is bolded.

Within the DLDwnrf group, statistically significantly better achievements of school-age children were observed on VS and PS assessment (VS – $F_{1;\ 28}$ = 19.991, $p \leq 0.000$; PS – Welch $F_{1;\ 28}$ = 20.386, $p \leq 0.000$), while statistically significant differences between preschoolers and schoolers were not found on the LPr assessment ($F_{1;\ 23}$ = 0.045, p = 0.833). In the TD group, statistically significantly better achievements of school-age children were observed on all three lexical tasks (VS – $F_{1;\ 39}$ = 9.110, p = 0.004; PS – $F_{1;\ 39}$ = 8.938, p = 0.005; LPr – Welch $F_{1;\ 16.182}$ = 7.016, p = 0.017).

Discussion

In this study, we examined three dimensions of expressive LS abilities by applying specific tests of EV assessment, PS, and the LPr in continuous speech. The results showed that both groups of DLD children differed significantly from their TD peers in all three dimensions of LS abilities. Regardless of the presence of NRF, DLD children have significantly poorer EV, sparse semantic networks, and difficulties in PS, and they use significantly fewer words in spontaneous speech compared to TD peers. A significantly lower number of correct answers on the naming test indicates a smaller volume of DLD children's vocabulary. Several previous studies have identified difficulties in naming in DLD children [42–44]. DLD children may even have a level of EV similar to children with autism spectrum disorder [45]. In terms of PS, our results show that all DLD children have significantly lower results compared to TD peers. Namely, a significantly lower number of mature associations shows that DLD children have deficits in organization and sparse LS networks. Our results confirm the results of several previous studies that examined PS in DLD children [12, 34, 46]. Additionally, both DLD groups have lower achievements compared to TD children in the domain of LPr, regardless of the presence of NRF. These results confirm the results of several previous studies of LPr in DLD children [13, 14, 47, 48].

The comparison analysis within the group of DLD children indicated certain specifics. In other words, DLDph children have significantly lower scores compared to DLDwnrf children on PS tasks but not on naming and LPr assessment. These results indicate a potential effect of PH, even in a mild form, on the severity of deficits in semantic network organization but not on VS and LPr in continuous speech. There are no studies in the literature that have analyzed the impact of PH on the severity of language deficit in DLD children for direct comparison, but there are a few that have examined the impact of perinatal RF on language outcome in the population of TD children. The influence of RF on the language abilities of preschoolers with speech and language disorders was analyzed in the study by Tomblin et al. [49]. The results of this study showed that children who experienced some of the prenatal or perinatal RF (infections, low birth weight, hypoxia) have lower scores at general language assessment, compared to children without pre/perinatal RF. Furthermore, Fox et al. [50] stated that, of the several RF studied, prenatal and perinatal RF are most associated with speech and language difficulties at a later age. One of the few studies that have examined the impact of prenatal and perinatal RF on children's achievement on specific language tests is a study by Duncan et al. [51], which confirmed a link between the presence of RF and poor performance on specific language assessment tests. Particularly, the mentioned study compared the achievements of prematurely born children (without the presence of cognitive deficits, sensory and intellectual disabilities) 4 to 7 years old to children without any perinatal complications. The results of this study showed a significant and negative impact of RF on the mean length of utterance, syntactic complexity, and short-term memory. Significantly lower achievements of PS in DLDph children can be explained by the possible presence of cognitive deficits. Namely, cognitive deficits are often observed in these children at a later age, without more pervasive cognitive impairment and with or without a history of HIE. Of the various cognitive deficits, pronounced memory deficits are the most common [52, 53]. On the other hand, PS is a domain of LS that is highly related to different dimensions of memory, including short-term memory, working memory, and cognitive processing speed [54, 55]. However, for reliable conclusions and implications for future research, the sample should be expanded, and tests for assessing specific cognitive abilities added.

A detailed analysis of the achievements in preschool and school-age children indicated specific developmental tendencies in all three dimensions of LA and semantic abilities. In other words, age proved to be a significant factor of improvement in DLDwnrf and TD children regarding PS. However, the comparison analysis of preschool and early school-age children's achievements showed that DLDph children progress only within EV skills, while DLDwnrf children progress significantly within EV and PS. On the other hand, a significant improvement in all assessed LA was observed in TD children. That means that DLDwnrf children progress significantly more than DLDph children within general LA. Given that both groups of DLD children have been covered with treatment in a specialized institution for a long period of time, we can assume that PH may pose a significant risk for more severe lexical deficits in DLD children, which may be quite resistant to conventional rehabilitation approaches used in treatment. There are two possible explanations for this. One is that even a milder form of hypoxia in DLD children can lead to comorbidity with specific cognitive deficits that cannot be detected with standard and general cognitive assessment. Assessing specific cognitive abilities that are highly related to LS abilities, such as working memory or cognitive processing speed, is not usually a part of general cognitive assessment. The obtained results may be explained by the possible comorbidities with a specific cognitive deficit but also by the fact that PH can contribute to significantly slower maturation of the brain and neural networks that underlie language abilities. Synaptic pruning is an important part of neural network formation that underlies speech and language abilities [56].

Namely, LPr is an ability that lies at the syntactic-semantic crossroads and, to some extent, depends on syntactic abilities. As syntactic deficit is often a dominant symptom in DLD children [2], it may significantly contribute to the non-

progression of both DLD groups. Reliable measurement of the semantic dimension of LPr in continuous speech should include measuring the LPr of only content words, such as nouns, adjectives, and verbs. That is one of the shortcomings of this study and its implications for future research.

Finally, we would like to state the most major limitation of the study. In general, DLD is a very heterogeneous disorder [2], which in such small clinical subgroups leads to frequent violations of the rules of sample homogeneity and normality of distribution, which limits the application of statistical measures with high reliability of the obtained results. A significantly higher number of children in subgroups would allow for more reliable conclusions, which is one of the implications for future research.

In order to better understand the NRF influence on the language outcome in DLD children, more research with language-specific tests is needed, which would also include phonological and syntactic abilities. Furthermore, future research should include tests for assessing specific cognitive abilities and their relationship with language skills.

Conclusion

PH in DLD children can lead to a more severe degree of LS deficit than these children would otherwise have. That is manifested with a more severe deficit of PS, which indicates a weaker organization and sparse LS network, otherwise underdeveloped in DLD children. However, a more extensive problem is that the PH presence in DLD children can cause significantly slower progress in all observed dimensions of LS abilities, even with language therapy. Slower progress was observed in the area of EV skils, semantic PS, and LPr in continuous speech. Given the immense importance of LS abilities in mastering academic skills, the DLDph children may have significantly more difficulties in that domain than DLDwnrf children.

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Current approaches to the treatment of metastatic brain tumors

Sadašnji pristupi lečenju metastatskih promena na mozgu

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Abstract

Background/Aim. About 10% of patients diagnosed with systemic cancer had brain metastases in the central nervous system. Patients with diseased lungs, breasts, urinary and digestive tract, as well as melanoma of the skin, are increasingly treated by neurosurgeons due to their dissemination and creation of secondary deposits in the brain. The aim of the study was to assess the efficacy of treatment modalities for patients with brain metastasis according to their type – solitary or multiple, and also to the primary cancer site. **Methods.** The retrospective study was performed at the Clinic for Neurosurgery and the Clinic for Oncology from 2018 to 2020. One hundred and eleven patients with solitary changes in the brain and 122 patients with multiple changes were examined. **Results.** It was found that multiple metastases were more common in primary lung cancer, while single metastases were more common in adenocarcinoma. However, patients with primary adenocarcinoma died in a significantly higher number. **Conclusion.** Surgery and radiation remain the cornerstone of therapy for symptomatic lesions. We should strive to improve surgical techniques in the direction of less damage to the surrounding healthy tissue. Radiosurgery, as well as whole brain radiotherapy, remains the basic form in the treatment of multiple metastases.

Key words:
brain neoplasms; mortality; neoplasm metastasis; neurosurgical procedures; radiotherapy; treatment outcome.

Apstrakt

Uvod/Cilj. Oko 10% bolesnika sa dijagnozom sistemskog karcinoma imalo je metastaze u mozgu u centralnom nervnom sistemu. Bolesnici sa obolelim plućima, dojkama, mokraćnim i digestivnim traktom, kao i oni sa melanomom kože, sve češće se, zbog širenja metastaza i stvaranja sekundarnih naslaga u mozgu, leče kod neurohirurga. Cilj rada bio je da se proceni efikasnost modaliteta lečenja bolesnika sa metastazama u mozgu prema njihovom tipu – solitarne ili multiple, kao i prema lokalizaciji primarnog karcinoma. **Metode**. Retrospektivna studija rađena je na Klinici za neurohirurgiju i Klinici za onkologiju u periodu od 2018. do 2020. godine. Pregledano je 111 bolesnika sa solitarnim promenama na mozgu i 122 bolesnika sa višestrukim promenama. **Rezultati.** Utvrđeno je da su višestruke metastaze bile češće kod obolelih od primarnog karcinoma pluća, dok su pojedinačne metastaze bile češće kod obolelih od adenokarcinoma. Međutim, preminuo je značajno veći broj bolesnika sa primarnim adenokarcinomom. **Zaključak.** Hirurgija i zračenje ostaju kamen temeljac terapije simptomatskih lezija. Potrebno je težiti unapređenju hirurške tehnike u pravcu što manjeg oštećenja okolnog zdravog tkiva. Radiohirurgija, kao i radioterapija celog mozga, ostaju osnovni oblici u lečenju višestrukih metastaza.

Ključne reči:
mozak, neoplazme; mortalitet; neoplazme, metastaze; neurohirurške procedure; radioterapija; lečenje, ishod.

Introduction

In addition to primary brain tumors, such as meningiomas, gliomas, and astrocytomas, recently, more and more people with metastatic changes, i.e., secondary lesions, have been contacting a neurosurgeon. About 10% of patients diagnosed with systemic cancer develop brain metastases in the central nervous system (CNS). The most common tumors that metastasize to the brain are lung, breast, kidney, and colon cancers [1]. Lung cancer metastases are the most common, even though the incidence of breast cancer is increasing, and melanoma represents a greater predisposition than all systemic types of cancer that lead to brain metastasis. As for breast cancer control, although different factors contribute to varying degrees in different countries, it is mainly due to increased breast awareness, early detection, and the provision of the most appropriate therapy to women with the disease are present [2]. About 16% of the

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world's population is covered by registration systems that provide cancer incidence statistics, while mortality data are available for about 29%. The incidence and mortality from breast cancer vary significantly depending on the world region [3]. Eighty percent of brain metastases are localized in the hemisphere, 15% are localized at the cerebellar level, and 5% at the bone level. Brain metastases are the most common neurological complication of systemic cancer and have a very poor prognosis. Lately, the management of patients with brain metastases has become more important due to the increased incidence of these tumors and the prolonged survival time of the patient that accompanies the increased control of systemic carcinoma [4]. Importantly, progress has been made in understanding molecular biology underlying the initial development and eventual proliferation of brain metastases [5].

The first step in fighting metastases is to discover the mechanism of their occurrence. It was previously thought that metastatic cancer cells attach to cells (neurons) of the grey and white masses of the brain. The researchers confirmed that in more than 95% of cases, cancer cells do not start growing in the brain tissue but on the walls of the blood vessels in the brain. British scientists have discovered how cancer cells attach to the blood vessels of the brain and lead to metastasis in this vital human organ. By studying the pathways of metastasis, a group of scientists found that cancer cells attach to the blood vessels of the brain using integrins, a proteins widely distributed in nature. Integrins enable contact of cells with surrounding tissues and the exchange of intercellular signals. Authors believe that removing integrins from the surface of cancer cells prevents tumor metastasis. With the development of drugs that block integrins, progress can be made in the fight against metastases [6].

Symptoms of brain tumors are manifested in the form of headaches that change depending on the time of day, weather changes, epi attacks, numbness, numbness or weakness of one half of the body, nausea accompanied by vomiting, memory loss, speech problem, and mood swings. Clinical examination with neurological examination and monitoring of patients with primary processes on organs with possible dissemination of secondary changes represents an initial step in the prevention of multiple dissemination. The precise diagnosis of the present secondary changes in the brain, as well as their number and size, are set after the magnetic resonance imaging (MRI) of the brain. A study by Aslan et al. [7] showed that the application of magnetic resonance spectroscopy and dynamic sensitive contrast parameters could help distinguish high-grade glioma from solitary metastasis in 97% of cases.

The fact is that more than half of all metastases occurred within three years [8], so we advise that follow-up be started in the early periods after diagnosis.

There are four main ways to treat brain cancer: radiation, i.e., radiotherapy (RT), surgery, treatment with cytostatics (chemotherapy), and immunotherapy, as well as a combination of all or any individual therapy. Treatment of brain tumors through immunotherapy is based on stimulating or establishing the ability of the immune system to fight infections and the progression of cancer cells [9].

Treatment options for patients with cerebral metastases are limited and mainly depend on the number and size of lesions and the progression of the primary metastatic disease [10].

With multiple changes, the use of the Gamma Knife® has greatly improved and advanced the treatment of these patients. Radiosurgical (RS) treatment of brain metastases results in survival times that are favorably compared to historical experience in patients treated with whole brain RT (WBRT) or surgical resection alone [11]. Indications for using a Gamma Knife® are that the changes are well limited, the size of the change does not exceed 35 mm, and there is no large perifocal edema. The dose usually used is from 16 to 140 Gy. Gamma Knife® surgery is widely used for a number of neurological disorders. However, little is known about its long-term complications, such as carcinogenic risks, as described in the study by Wang et al. [12].

The problem is the secondary changes of larger dimensions, which are not subject to Gamma Knife® treatment. Then the methods of combined treatment are resorted to. If the patient's condition is satisfactory and the primary process is in remission, a large secondary deposit surgery is performed first, followed by RT.

There is no way to know exactly how long someone will live with brain metastases. It depends on many factors, including the type of primary process, the number of brain tumors, and the treatments used. The median and one-year survival rates initially in patients with metastases were ten months and 41%, respectively. The median time to metastasis in patients with the localized disease was 28 months [8].

Therefore, as we continue to strive for better access to and advancement in technology, improved survival in these settings should be achieved through increased awareness of cancer and its potential for successful treatment [13].

Methods

The retrospective study was carried out at the Clinic for Neurosurgery and the Clinic for Oncology, University Clinical Center Niš, Serbia, from 2018 to 2020. One hundred and eleven patients with solitary changes in the brain and 122 patients with multiple changes were examined. In all patients, in addition to multislice computed tomography (MSCT) of the brain or MRI of the brain, MSCT of the lungs and echocardiography or MSCT of the abdomen were performed. Patients with a solitary change were treated at the Clinic for Neurosurgery using operative treatment. Patients with multiple brain changes, who were not indicated for surgical treatment, were referred to the Clinic for Oncology, where they were treated with RT and chemotherapy. Patients with multiple changes, small diameter, good general condition, and remission of the primary disease were treated with Gamma Knife® at the Institute of Neuroradiology Belgrade.

Statistical analysis

Qualitative data were expressed as frequencies and percentages, while quantitative data were presented as mean and standard deviations. The normality of distribution was tested by the Kolmogorov-Smirnov test. Variables were compared by a two-tailed Student's *t*-test for continuous variables for normal distribution or the Mann-Whitney *U* test for continuous variables of non-normal distribution. The chi-square (χ^2) test and Fisher test were used for categorical variables. Statistical analyses were performed using the statistical software SPSS Statistics 22.0 (SPSS, Inc., Armonk, NY, USA). All *p*-values presented were 2-tailed, and $p < 0.05$ was considered statistically significant.

Results

In the three-year period (2018–2020), there were 111 patients with one operated metastasis in the examined population, among whom there were 70 (63.1%) men and 41 (36.9%) women. There was no significant difference in age structure by sex ($p = 0.071$).

A total of 9 patients died, of whom 7 (77.8%) were men and 2 (22.2%) women, but without statistical significance ($p = 0.340$).

There was no significant difference in the distribution of patients by sex according to the examined years ($p = 0.100$) or age structure ($p = 0.156$). In relation to the distribution of metastasis localization, there was no statistically significant difference in the three examined years. The number of deceased patients by age was equal without significant difference ($p = 0.984$) (Table 1).

There was also no statistically significant difference in the distribution of exitus in individual metastases, depending on the localization of the primary process ($p = 0.216$) (Table 2).

In the examined group in the three years, there were 122 patients with multiple metastases, among which there were 68 (55.7%) men and 54 (44.3%) women. There was no significant difference in age structure by sex ($p = 0.480$).

There was no significant difference in the distribution of patients by sex according to the examined years ($p = 0.202$) nor by age structure ($p = 0.698$). In relation to the distribution of localization of primary cancer, there was no statistically significant difference in the three examined years ($p = 0.501$). The number of deceased patients by age was equal without significant difference ($p = 0.744$) (Table 3).

In patients with multiple brain metastases, a significant difference in the number of deaths was found depending on the localization of primary cancer ($\chi^2 = 11.816$; $p = 0.037$). Patients with primary carcinoma of the digestive tract died in a significantly higher number ($p = 0.037$) (Table 4).

It was also shown that a significantly higher number of deceased patients were treated with RT than with the surgical approach ($\chi^2 = 33.213$; $p < 0.001$) (Table 5).

A significant difference in the existence of single or multiple metastases depending on the primary process was found ($\chi^2 = 35.041$; $p < 0.001$). Multiple metastases are more common in primary lung cancer, while single metastases are more common in digestive tract carcinoma (Table 6).

A significant difference in the distribution of metastatic changes in the three years was also observed ($\chi^2 = 11{,}768$; $p = 0.019$). Oligometastases were more prevalent in 2020, while multiple metastases were present in 2018 (Table 7).

Table 1

Data according to the years of examination of patients with one brain metastasis

Parameter	2018	2019	2020	*p*-value
Gender				
m	17 (48.6)	28 (70.0)	25 (69.4)	0.100
f	18 (51.4)	12 (30.0)	11 (30.6)	
Age (years)	65.74 ± 7.61	65.40 ± 10.46	61.50 ± 12.30	0.156
Localization				
digestive tract	7 (20.0)	13 (32.5)	14 (38.9)	
kidney	0 (0.0)	1 (2.5)	2 (5.6)	
breast	11 (31.4)	7 (17.5)	5 (13.9)	0.345
melanoma	4 (11.4)	2 (5.0)	4 (11.1)	
lungs	13 (37.1)	17 (42.5)	11 (30.6)	
Exitus	3 (8.6)	3 (7.5)	3 (8.3)	0.984

m – male; f – female. All results are given as number (percentage) except age which is shown as mean ± standard deviation.

Table 2

Distribution of the deceased according to the localization of the primary cancer

Localization	Deceased patients		*p*-value
	no	yes	
Digestive tract	32 (94.1)	2 (5.9)	
Kidney	2 (66.7)	1 (33.3)	
Breast	23 (100.0)	0 (0.0)	0.216
Melanoma	9 (90.0)	1 (10.0)	
Lungs	36 (87.8)	5 (12.2)	

All values are expressed as numbers (percentages).

Table 3

Data according to the years of examination of patients with multiple metastases

Parameter	2018	2019	2020	*p*-value
Gender				
m	34 (55.7)	19 (47.5)	15 (71.4)	0.202
f	27 (44.3)	21 (52.5)	6 (28.6)	
Age (years)	63.28 ± 11.17	64.85 ± 11.12	62.33 ± 11.84	0.698
Primary digestive tract carcinoma	2 (3.3)	2 (5.0)	2 (9.5)	
Localization of primary cancer				0.501
breast	8 (13.1)	6 (15.0)	2 (9.5)	
liver	1 (1.6)	0 (0.0)	0 (0.0)	
melanoma	5 (8.2)	2 (5.0)	5 (23.8)	
lungs	43 (70.5)	28 (70.0)	12 (57.1)	
urinary	2 (3.3)	2 (5.0)	0 (0.0)	
Therapy				
gamma	28 (45.9)	14 (35.0)	8 (38.1)	0.529
radiant	33 (54.1)	26 (65.0)	13 (61.9)	
Exitus	20 (32.8)	16 (40.0)	8 (38.1)	0.744

m – male; f – female. All results are given as number (percentage) except age which is shown as mean ± standard deviation.

Table 4

Distribution of deaths by localization of primary cancer in patients with multiple brain metastases

Localization	Deceased patients		*p*-value
	no	yes	
Digestive tract	1 (1.3)	5 (11.4)	0.037
Breast	14 (17.9)	2 (4.5)	ns
Liver	0 (0.0)	1 (2.3)	ns
Melanoma	8 (10.3)	4 (9.1)	ns
Lungs	53 (67.9)	30 (68.2)	ns
Urinary	2 (2.6)	2 (4.5)	ns

ns – non significant. All values are expressed as numbers (percentages).

Table 5

Distribution of the deceased according to the type of therapy

Therapy type	Deceased patients		*p*-value
	no	yes	
RT	47 (60.3)	3 (6.8)	< 0.001
WBRT	31 (39.7)	41 (93.2)	

RT – radiotherapy; WBRT – whole brain RT. All values are expressed as numbers (percentages).

Table 6

Distribution of localization of primary cancers

Localization	Single metastases	Multiple metastases	*p*-value
Lungs	41 (36.9)	83 (68.6)	
Breast	23 (20.7)	16 (13.2)	
Melanoma	10 (9.0)	12 (9.9)	< 0.001
Urinary	2 (2.7)	4 (3.3)	
Digestive tract	34 (30.6)	6 (5.0)	

All values are expressed as numbers (percentages).

Table 7

Distribution of metastatic changes according to the years of examination

Metastases type	2018	2019	2020	*p*-value
Single	35 (37.8)	40 (35.1)	36 (45.6)	0.019
Multiple	61 (48.4)	40 (35.1)	21 (26.6)	

All values are expressed as numbers (percentages).

Discussion

About 10% of patients diagnosed with systemic cancer have brain metastases in the CNS. Diseased lungs, breasts, urinary and digestive tract, as well as melanoma of the skin, are increasingly treated by neurosurgeons due to their dissemination and creation of secondary deposits on the brain. Lungs, breasts, and skin (melanoma) are the most common sources of brain metastases, and up to 15% of patients' primary site remains unknown [14]. Early diagnosis and suspicion of this dissemination in primary metastases enable surgical intervention followed by oncological treatment, which, together, prolong the patient's life. The average survival time with brain metastases is usually less than a year. However, when only isolated metastases (oligometastases) are found and are able to be treated, over 60% of people have the chance of surviving two years or longer [15].

In our study, in the three-year period (2018–2020) in the examined population, there were 111 patients with one operated metastasis, among which there were 70 (63.1%) men and 41 (36.9%) women. There was no significant difference in the age structure according to gender or concerning the existence of the primary process in the examined period. The optimal management of brain metastases remains controversial. WBRT and local treatment or surgery or RS are the cornerstones of treatment. Combination treatment can improve both overall survival and local control in patients with a single metastasis but also leads to the benefit of local control in patients with two to four lesions [16].

In patients with primary metastases caused by adenocarcinomas, it often happened that the patient underwent surgery for a change in the brain of unknown etiology and that the pathohistological (PH) finding gave us guidance in further examination. Before neurosurgical treatment, such patients were not treated by a gastroenterologist, but their treatment in that direction began only after the obtained PH findings. In the last few decades, surgical resection has evolved into a standard treatment that has led to improved clinical outcomes in carefully selected patients with brain metastases [17].

Fatal outcomes occurred in nine patients, with no significant differences in the distribution of exitus according to the location of metastases (p = 0.216). Kanner et al. [18] believe that aggressive intervention may be indicated for selected patients with well-controlled systemic cancer and good performance status in whom CNS disease poses the greatest threat to functionality and survival. Furthermore, Kuo and Recth [19] believe that an aggressive therapeutic approach for at-risk patients, which includes a combination of either surgery or stereotactic RS and WBRT, can improve survival and reduce the risk of CNS progression.

In the same period, 122 patients with multiple metastases were treated, among whom 68 (55.7%) were men and 54 (44.3%) women. Here, too, we did not have a significant statistical difference in terms of age structure, sex, and distribution of primary processes. Patients with primary carcinoma of the digestive tract died in significantly higher numbers. Kocher et al. [20] believe RS in patients with one to three cerebral metastases leads to significant survival benefits only in younger patients with low systemic tumor load compared with WBRT alone.

The results of treated patients with multiple metastases showed that a higher number of patients who received standard RT (WBRT) died than those patients treated with Gamma Knife® (RS). That can be explained by the fact that patients in better grades (Karnofski scale used) were referred to Gamma Knife® therapy, along with younger patients, compared to patients treated with standard radiation therapy. Métellus et al. [21] suggest that therapeutic decision is subject to multidisciplinary analysis, taking into account established prognostic factors, including general patient status, extracerebral disease status, and clinical and radiological presentation of lesions.

The study showed that multiple metastases were more prevalent in primary lung cancer, while single metastases were more prevalent in carcinoma of the digestive tract. A study by Hoffman et al. [22] showed that RS is an effective therapy for selected patients with newly diagnosed or recurrent brain metastases from lung cancer. Since the fatal outcome was most prevalent in patients with multiple brain metastases where carcinoma of the digestive tract was the primary process, we assume that the diagnosis of carcinoma of the digestive tract is much more difficult to come by. Attempts to detect a primary tumor are most often in vain, however, because its origin is established in only 13% of patients, regardless of diagnostic methods used [23]. The symptoms of the gastrointestinal tract are very non-specific, patients do not recognize them often, and only when the disease has progressed is the right diagnosis made. Advanced adenocarcinoma causes multiple changes in the brain, so the applied therapy does not ensure satisfactory success, and the patient quickly reaches a lethal outcome. These patients are treated with WBRT. Sahgal et al. [24] believe that WBRT is associated with toxicity that may influence the decision to use WBRT, and, therefore, acute side effects of WBRT and more serious late side effects of neurocognitive impairment and leukoencephalopathy should be considered. Hepatocellular carcinoma is the most common primary malignancy of the liver and the third leading cause of cancer-related death [25].

Regarding the three-year cross-section of treated patients at the Clinic for Neurosurgery in Niš, it was noticed that multiple changes were more often diagnosed during 2018, and individual metastases were more common in 2020. That supports the fact that the observation and follow-up of patients with primary cancers are more adequate and better. Modern diagnostic procedures, such as the use of MRI, have made it possible for changes in the brain to be detected earlier and, as individual secondary deposits in the brain, to be treated surgically. Sharma et al. [26] suggest improving primary healthcare, especially when it comes to breast cancer, believing the mortality rate would then decrease and the survival period would greatly increase.

From the aforementioned, we see that the optimal treatment of brain metastases is still controversial, although there is a clear role of RT in the treatment of this disease. Specific treatment recommendations depend on

different clinical parameters and patient preferences. Consistently, multiple studies of WBRT have shown improved local control and reduced progression elsewhere in the brain but no overall survival benefit and concern for the poorer neurocognitive outcome. However, both new drugs and technological advances in the provision of RT aim to reduce the neurocognitive consequences of WBRT. Stereotactic RS has been shown to improve overall survival in patients with a single brain metastasis compared with WBRT [27].

Conclusion

Treatment options for patients with cerebral metastases are limited and mainly depend on the number and size of lesions as well as the degree of evolution of the primary disease. Surgery and radiation remain the cornerstone of therapy for symptomatic lesions. We should strive to improve surgical techniques in the direction of causing less damage to the surrounding healthy tissue. RS, as well as WBRT, remain the basic form in the treatment of multiple metastases.

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Translation, transcultural adaptation, and validation of the Serbian version of the PSS-QoL questionnaire – a pilot research

Prevod, transkulturalna adaptacija i validacija srpske verzije upitnika PSS-QoL – pilot istraživanje

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Abstract

Background/Aim. The assessment of health-related quality of life (HRQoL) is fundamental for a better understanding of the effect of a disease on different aspects of a patient's daily functioning and the efficacy of the treatment modalities. Primary Sjögren's Syndrome (SS) Quality of Life Questionnaire (PSS-QoL) is the first disease-specific instrument for evaluating HRQoL in patients with primary SS. The aim of this study was to formally translate the PSS-QoL questionnaire from English to Serbian, assess its psychometric properties, and validate it for use in the Serbian population. **Methods.** The research was designed as a pilot study and included 30 participants. Internal consistency was determined by calculating Cronbach's alpha coefficient. The construct validity of the questionnaire was estimated by the correlation of its overall result with the patients' EuroQoL-5D, European League Against Rheumatism (EULAR) SS Patient Reported Index, Oral Health Impact Profile-14, and Emotion Regulation Questionnaire scores. **Results.** There were 29 (96.7%) female participants and one (3.3%) male participant in the research. The average (± standard deviation) score of PSS-QoL was 44.63 ± 12.901 at baseline and 41.70 ± 12.075 at follow-up. Cronbach's alpha value of the Serbian version of PSS-QoL was 0.922. The test-retest intraclass correlation coefficient was 0.981 (95% confidence interval: 0.436–0.996). Analysis revealed a statistically significant moderate to strong correlation between PSS-QoL scores and EuroQol-5D (r_s = -0.696), EULAR SS Patient Reported Index (r_s = 0.883), and Oral Health Impact Profile-14 scores (r_s = 0.809). **Conclusion.** Serbian adaptation of the PSS-QoL instrument can be used to evaluate HRQoL of patients with primary SS both in academic research and clinical practice as a novel outcome measure.

Key words:
quality of life; serbia; sjogren's syndrome; surveys and questionnaires; translations.

Apstrakt

Uvod/Cilj. Procena kvaliteta života povezanog sa zdravljem – *health-related quality of life* (HRQoL), je esencijalna za bolje razumevanje uticaja bolesti na različite aspekte svakodnevnog funkcionisanja bolesnika i efikasnost modaliteta lečenja. Upitnik primarnog Sjegrenovog sindroma (SS) – *Primary Sjögren's Syndrome QoL* (PSS-QoL), je prvi instrument za procenu HRQoL koji je specifičan za primarni SS. Cilj rada bio je da se upitnik PSS-QoL prevede sa engleskog na srpski jezik, procene njegova psihometrijska svojstva i da se validira za upotrebu u srpskoj populaciji. **Metode**. Istraživanje je osmišljeno kao pilot studija i obuhvatilo je 30 učesnika. Interna konzistencija izračunata je određivanjem Kronbahovog alfa koeficijenta. Valjanost upitnika procenjena je korelacijom njegovog ukupnog rezultata sa rezultatima *EuroQoL-5D*, *European League Against Rheumatism (EULAR) SS Patient Reported Index*, *Oral Health Impact Profile-14* i *Emotion Regulation Questionnaire* upitnika. **Rezultati.** U istraživanju je učestvovalo 29 (96,7%) žena i jedan (3,3%) muškarac. Prosečna (± standardna devijacija) vrednost PSS-QoL bila je 44,63 ± 12,901 na početku i 41,70 ± 12,075 prilikom ponovnog merenja. Vrednost Kronbahove alfa vrednosti za srpsku verziju PSS-QoL bila je 0,922. Test-retest koeficijent korelacije iznosio je 0,981 (95% interval pouzdanosti: 0,436–0,996). Analiza je otkrila statistički značajnu, umerenu do jaku korelaciju, između PSS-QoL rezultata i *Euro-QoL-5D* (r_s = -0,696), *EULAR SS Patient Reported Index* (r_s = 0,883) i *Oral Health Impact Profile-14* rezultata (r_s = 0,809). **Zaključak.** Srpska verzija instrumenta PSS-QoL može se koristiti za procenu HRQoL kod bolesnika sa primarnim SS, kako u akademskim istraživanjima tako i u kliničkoj praksi.

Ključne reči:
kvalitet života; srbija; sjegrenov sindrom; ankete i upitnici; prevodilaštvo.

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Introduction

Primary Sjögren syndrome (SS) is an autoimmune rheumatic disease characterized by intense lymphocytic infiltration of exocrine glands, which results in their progressive and irreversible dysfunction [1]. It predominantly affects women in the 4th and 5th decade of life [2]. The main symptoms associated with primary SS are oral and ocular dryness, fatigue, and chronic pain [3]. In approximately 25–50% of patients, severe systemic features, including musculoskeletal, pulmonary, hematological, and neurological complications, might be present alongside glandular manifestations [4, 5]. Mental disorders, such as anxiety and depression, are also frequently reported [6]. It is suggested that both physical and psychological factors contribute to the decrease in the overall well-being of patients suffering from primary SS [7].

Health-related (HR) quality of life – QoL (HRQoL) is a multidimensional concept used as a patient-reported outcome measure in numerous clinical trials [8]. The assessment of HRQoL is fundamental for a better understanding of the effect of a disease on different aspects of a patient's daily functioning, the efficacy of the treatment modalities, and possible risk factors related to altered HRQoL [9, 10]. Over the years, various generic and disease-specific instruments have been developed and translated into different languages worldwide for HRQoL measurement.

Evaluation of HRQoL in SS has been, so far, mostly accomplished by implementing generic tools such as 36-Item Short Form Survey (SF-36) and EuroQol-5D (EQ-5D) [11]. On the other hand, primary SS QoL (PSS-QoL) questionnaire represents the first created disease-specific instrument for HRQoL assessment of patients with primary SS [9]. It consists of 25 questions divided into two dimensions – physical (dryness and discomfort) and psychosocial. The overall score of the questionnaire is obtained by simply adding the values of the items, and it may range from 0 to 92 for males and from 0 to 96 for females [9]. Higher results indicate a greater impairment of HRQoL.

The aim of this research was to formally translate the PSS-QoL questionnaire from English to Serbian, assess its psychometric properties, and validate it for use in the Serbian population.

Methods

The study was conducted on the premises of the Rheumatology Clinic of the University Clinical Center of Kragujevac between July 5 and December 15, 2021, and it was designed as a pilot study. The research was approved by the Ethics Committee of the University Clinical Center of Kragujevac (No. 01/20-657, from September 09, 2020). Before participating in the study, the aim and the protocol were explained to patients, and all of them signed the written informed consent. Thirty patients with primary SS that came to a regular checkup at the Rheumatology Clinic were recruited in the research. The study included patients with the diagnosis of primary SS according to the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification recommendations and those aged 18 or above. The exclusion criteria were the following: age below 18, mental disorders, and patients unwilling to participate in the study. All of the patients filled in the following questionnaires: PSS-QoL, EuroQol-5D (EQ-5D), EULAR SS Patients Reported Index (ESSPRI), Oral Health Impact Profile-14 (OHIP-14), and Emotion Regulation Questionnaire (ERQ).

HRQoL of patients with primary SS was evaluated using the Serbian version of the PSS-QoL questionnaire, which was translated, adapted, and validated in this paper. Translation and cultural adaptation of the PSS-QoL questionnaire were made according to the standard protocol (translation/back-translation), following the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines [12]. The author of the original instrument (Dr. Angelika Lackner [9], Medizinische Universität Graz) provided written approval for translation and adaptation from English to the Serbian language. The original version of the questionnaire was first translated into Serbian independently by two authors of this article, whose native language is Serbian. After the translation was done, the authors compared the two versions and combined them, making minor wording adjustments to retain the linguistic properties of the Serbian language. The questionnaire was then back-translated into English by a proficient English speaker and a fluent Serbian speaker who had not been familiar with the original version. The back-translation was compared with the original, and the authors of the article agreed on the final Serbian version of the instrument. In order to test the psychometric properties of the PSS-QoL instrument, 30 patients completed the questionnaire twice in 14 days.

The PSS-QoL questionnaire is the first disease-specific instrument designed to evaluate the HRQoL of patients with primary SS [9]. It is composed of 25 questions divided into physical and psychosocial dimensions. All questions refer to patients' complaints in the last four weeks. The physical domain consists of a numeric scale (from 0 to 10) for pain assessment and checkboxes for other disease-related physical symptoms that are known to alter HRQoL. The psychosocial dimension includes 14 items that can be scored on a 5-point Likert scale (from 0 – never to 4 – always). Statements 15 and 20 within the psychosocial domain have inverse scoring. The total score is calculated as a summary of individual items, and it may range from 0 to 96 (for females) and from 0 to 92 (for males, vaginal dryness is excluded). Higher scores indicate worse HRQoL [9].

EQ-5D is a reliable, self-completion HRQoL instrument used in many various diseases and health conditions [13]. It is the most widely implemented generic tool worldwide. EQ-5D consists of five domains – mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, and each of these dimensions has five levels of severity. EQ-5D also includes a visual analog scale (EQ-VAS) that provides individuals' perception of their general health, recorded on a vertical scale, numbered from 0 to 100 [13]. The final score of

the questionnaire is calculated according to set values specific to different countries.

ESSPRI is a patient-reported outcome measure created to evaluate the main symptoms associated with primary SS, such as overall dryness, fatigue, and body pain. It contains three numeric scales (from 0 – no symptom to 10 – worst symptom imaginable) to assess symptoms' severity in the last two weeks. The overall result represents a mean value of the three domains' scores, ranging from 0 to 10 [14]. So far, ESSPRI has been widely used as an endpoint in clinical research because it is considered a useful predictor of the health status of primary SS patients and correlates well with HRQoL domains as well [15].

OHIP-14 is a self-filled questionnaire that consists of 14 questions divided into seven dimensions: functional limitation, pain, psychological discomfort, physical disability, psychological disability, social disability, and handicap [16]. It is created to measure the impact of oral health on the overall QoL domains. Questions can be scored on a 5-point Likert scale, depending on how much a certain oral HR problem affects the patient's daily functioning. The final result is obtained by summing the 14 items' values, and the higher scores indicate a negative impact of oral conditions on HRQoL [16].

ERQ is a 10-item scale designed to assess individual differences in emotion regulation using two common strategies: cognitive reappraisal and emotion suppression [17]. Answers can be scored on a 7-point Likert scale (from 1 – strongly disagree to 7 – strongly agree). Questions numbered 1, 3, 5, 7, 8, and 10 belong to the cognitive reappraisal domain, while questions 2, 4, 6 and 9 contribute to emotion suppression. A score is calculated for each of the subscales, with the higher scores indicating greater utilization of that particular emotion regulation strategy [17, 18].

Statistical data analysis was performed in the SPSS statistical program, version 22. The feasibility was determined by measuring participants' time for completing the PSS-QoL questionnaire and establishing the percentage of missing data for each question. The reliability of the Serbian version of the PSS-QoL was tested threefold. First, the internal consistency was estimated by calculating Cronbach's alpha (Cα) coefficient for the whole instrument. The internal consistency was considered satisfactory if the Cα coefficient was 0.7 or higher [12]. Second, the questionnaire was divided into two parts by the split-half method (even-numbered and odd-numbered questions), and Cα was determined for each of them, after which the Spearman-Brown coefficient was calculated. Third, the intraclass correlation coefficient (ICC) was evaluated for the questionnaire's test-retest reliability (temporal stability). Values of ICC above 0.7 indicated good reliability. To discover the principal factors of the Serbian version of the PSS-QoL instrument, the Principal Component Analysis (PCA) was performed, including only questions belonging to the psychosocial domain of the questionnaire (items in the physical dimension are not scored on a Likert scale). Prior to the PCA, the Kaiser-Meyer-Olkin (KMO) measure of Sampling Adequacy and Bartlett's test of sphericity were conducted to assess the sample suitability for this method. Then the factors were extracted based on Eigenvalues (> 1.0), scree-plot, and factor loading (> 0.3) [19]. The construct validity of the questionnaire was explored by the correlation of its overall result with the patients' EQ-5D, ESSPRI, OHIP-14, and ERQ scores, using Spearman's rank correlation. A *p*-value less than 0.05 was considered a measure of statistical significance for all statistical tests.

Results

A total of 30 primary SS patients with a mean [± standard deviation (SD)] age of 63.93 ± 10.48 years were included in the research. The average (± SD) disease duration was 111.200 ± 141.433 months. The sociodemographic characteristics of the study participants are given in Table 1.

Table 1

Sociodemographic characteristics of the study participants

Variable	n (%)
Gender	
male	1 (3.3)
female	29 (96.7)
Education	
elementary school	9 (30.0)
high school degree	11 (36.7)
university degree	7 (23.3)
doctorate	3 (10.0)
Employment status	
employed	3 (10.0)
unemployed	9 (30.0)
retired	18 (60.0)
Marital status	
single	1 (3.3)
married	20 (66.7)
divorced	7 (23.3)
widowed	2 (6.7)

The following scores of the PSS-QoL questionnaire were obtained: total PSS-QoL score (all questions), physical PSS-QoL (questions 1–11), discomfort PSS-QoL (questions 1–6), dryness PSS-QoL (questions 7–11), and psychosocial PSS-QoL (12–25) score. These results were calculated at baseline (total score: 44.630 ± 12.901) and two weeks after (total score: 41.700 ± 12.075) for the second evaluation of the questionnaire's psychometric properties.

Table 2 shows the scores of the EQ-5D, ESSPRI, OHIP-14, and ERQ questionnaires, including the results of their specific domains.

Feasibility

The feasibility of the questionnaire was satisfactory as there were no missing data, both at baseline and follow-up. The mean time for completing the questionnaire was 2.42 min (range from 1.41 to 3.23 min). The participants felt the questionnaire was easy to complete and the questions were relevant to their health status.

Reliability

The internal consistency of the questionnaire was assessed by calculating the Cα coefficient. The Serbian version of the PSS-QoL instrument recorded high reliability (Cα = 0.922).

After dividing the instrument into two parts by the split-half method, the Spearman-Brown coefficient was determined by the Spearman-Brown 'prophecy' formula and recorded a value of 0.972. Considering that the Spearman-Brown coefficient did not fall below 0.7 after the split-half method, the satisfactory reliability of the Serbian version of the PSS-QoL questionnaire was further verified.

All of the recruited patients filled out the questionnaire once again after two weeks for test-retest reliability evaluation (100% response rate). The value of ICC was 0.981 [95% confidence interval (CI): 0.436–0.996], which indicated excellent reliability of the PSS-QoL instrument.

The mean values of total and individual domain scores of PSS-QoL at baseline and follow-up are given in Table 3.

Principal Component Analysis

Considering that the KMO measure of Sampling Adequacy was 0.765 and Bartlett's test of sphericity was significant ($p < 0.001$), PCA could be performed. Only one component was extracted (Eigenvalue = 8.343), explaining in total 59.592% of the variance. All of the items had high values of nonrotating factor weights (> 0.3; range from 0.667 to 0.932) for one component (Table 4). The scree-plot also suggested a one-factor solution. Therefore, the rotation was not conducted.

Construct validity

The correlation of the PSS-QoL scores with the scores of other similar HRQoL instruments (EQ-5D, ESSPRI,

Table 2

EQ-5D, ESSPRI, OHIP-14, ERQ scores

Instrument	Values, mean ± SD; median (IQR)
EQ-5D	
mobility	1.37 ± 0.808; 1.0 (1.0)
self-care	1.37 ± 0.808; 1.0 (1.0)
usual activity	1.53 ± 0.808; 1.0 (1.0)
pain/discomfort	2.53 ± 0.819; 3.0 (1.0)
anxiety/depression	2.77 ± 0.935; 3.0 (1.25)
score	0.74 ± 0.318; 0.83 (0.2)
ESSPRI	
dryness	5.33 ± 1.539; 5.0 (2.25)
fatigue	4.50 ± 2.224; 5.0 (3.0)
pain	3.83 ± 1.783; 4.0 (2.0)
score	4.67 ± 1.728; 5.0 (3.0)
OHIP-14	
functional limitation	2.53 ± 1.106; 2.5 (1.0)
physical pain	5.40 ± 1.113; 5.5 (1.0)
psychological discomfort	2.77 ± 1.135; 3.0 (2.0)
physical disability	6.27 ± 1.015; 6.0 (1.0)
psychological disability	2.70 ± 1.179; 2.5 (2.0)
social disability	2.07 ± 0.980; 2.0 (2.0)
handicap	3.23 ± 1.040; 3.0 (2.0)
score	24.97 ± 5.586; 26.5 (10.0)
ERQ	
cognitive reappraisal	24.15 ± 2.509; 24.0 (4.0)
expressive suppression	20.65 ± 4.345; 22.0 (4.0)
score	32.36 ± 20.847; 42.0 (47.0)

EQ-5D – Euro Quality of life-5D; ESSPRI – European League Against Rheumatism (EULAR) Sjögren's Syndrome Patients Reported Index; OHIP-14 – Oral Health Impact Profile -14; ERQ – Emotion Regulation Questionnaire; SD – standard deviation; IQR – interquartile range.

Table 3

Mean values of PSS-QoL dimensions and total score at baseline and follow-up

PSS-QoL	Baseline (mean ± SD)	Follow-up (mean ± SD)
Score	44.63 ± 12.901	41.70 ± 12.075
Physical	14.73 ± 5.483	15.73 ± 5.644
Discomfort	5.00 ± 2.579	5.23 ± 2.541
Dryness	9.73 ± 3.542	10.50 ± 3.785
Psychosocial	27.40 ± 7.959	25.97 ± 7.434

PSS-QoL – Primary Sjögren's Syndrome Quality of Life; SD – standard deviation.

Table 4

Component matrix

Items	Factor weights
I have a feeling that I am the only person with these complaints	0.773
I have a feeling that my complaints are not taken seriously	0.785
I have a feeling that my complaints are too much for me	0.704
I have a feeling that my family and friends are understanding	0.691
I am too tired to fulfill obligations to my family and friends	0.774
I am withdrawn	0.678
I am concerned about the side effects	0.853
I worry about the further course of my disease	0.853
I have a good feeling about my body	0.679
I cannot manage my everyday life as well as I did before I became ill	0.932
I tire easily	0.841
Everyday activities like driving, work, household, and sports are a challenge	0.731
Remedies like eye drops, creams, and physiotherapy impose a financial burden	0.667
The disease has reduced my quality of life	0.873

Extraction Method: Principal Component Analysis; One component extracted.

Table 5

Correlation of PSS-QoL scores with EQ-5D, ESSPRI, and OHIP-14 scores

	PSS-QoL				
	score	physical	discomfort	dryness	psychosocial
EQ-5D					
mobility	n.s.	n.s.	n.s.	n.s.	n.s.
self-care	n.s.	n.s.	n.s.	n.s.	n.s.
usual activity	n.s.	n.s.	n.s.	n.s.	n.s.
pain/discomfort	0.812**	0.873**	0.926**	0.635**	0.649**
anxiety/depression	0.666**	0.678**	0.656**	0.569**	0.646**
score	-0.696**	-0.720**	-0.701**	-0.613**	-0.636**
ESSPRI					
dryness	0.829**	0.818**	0.742**	0.713**	0.696**
fatigue	0.814**	0.735**	0.711**	0.611**	0.761**
pain	0.832**	0.876**	0.965**	0.634**	0.653**
score	0.883**	0.863**	0.866**	0.702**	0.739**
OHIP-14					
functional limitation	0.488**	0.435*	0.535**	n.s.	0.465**
physical pain	0.728**	0.721**	0.679**	0.638**	0.644**
psychological discomfort	0.659**	0.654**	0.660**	0.511**	0.581**
physical disability	0.599**	0.502**	0.415*	0.475**	0.558**
psychological disability	0.697**	0.683**	0.725**	0.461*	0.705**
social disability	0.507**	0.515**	0.656**	n.s.	0.422*
handicap	0.692**	0.692**	0.711**	0.550**	0.618**
score	0.809**	0.790**	0.821**	0.618**	0.718**

PSS-QoL – Primary Sjögren's Syndrome Quality of Life; EQ-5D – Euro Quality of life-5D; ESSPRI – European League Against Rheumatism (EULAR) Sjögren's Syndrome Patients Reported Index; OHIP-14 - Oral Health Impact Profile – 14.
n.s. – not significant; * $p < 0.05$; ** $p < 0.001$.

OHIP-14) was evaluated to assess the questionnaire's convergent validity (Table 5). PSS-QoL discomfort correlated very strongly with the same EQ-5D dimension. Furthermore, the psychosocial component of PSS-QoL had a moderate correlation with the EQ-5D anxiety/depression domain. Results revealed a moderate negative correlation between the total PSS-QoL and EQ-5D scores.

The PSS-QoL questionnaire correlated strongly with all of the ESSPRI dimensions. There was a very strong correlation between ESSPRI pain and PSS-QoL discomfort, and also a strong correlation between ESSPRI dryness and PSS-QoL dryness.

Regarding PSS-QoL and OHIP-14, the results showed a very strong correlation between their total scores. OHIP-14 psychological disability and PSS-QoL psychosocial domain recorded a strong correlation.

The divergent validity of the PSS-QoL questionnaire was established by correlating its overall score with the values of the ERQ. The obtained results indicated a weak and non-significant correlation (r_s = -0.137) between these two instruments.

Spearman's correlation coefficients are shown in the Multitrait-Multimethod matrix (Table 6).

Table 6

Multitrait-multimethod correlation matrix

	PSS-QoL					EQ-5D	ESSPRI	OHIP-14	ERQ
	score	physical	discomfort	dryness	psychosocial				
PSS-QoL									
score	‘1								
physical	0.941**	1							
discomfort	0.818**	0.855**	1						
dryness	0.853**	0.902**	0.587**	1					
psychosocial	0.869**	0.725**	0.624**	0.640**	1				
EQ-5D	-0.696**	-0.720**	-0.701**	-0.613**	-0.636**	1			
ESSPRI	0.883**	0.863**	0.866**	0.702**	0.739**	-0.854**	1		
OHIP-14	0.809**	0.790**	0.821**	0.618**	0.718**	-0.741**	0.848**	1	
ERQ	-0.137	-0.195	-0.187	-0.187	-0.217	0.383*	-0.210	-0.121	1

PSS-QoL – Primary Sjögren’s Syndrome Quality of Life; EQ-5D – Euro Quality of life-5D; ESSPRI – European League Against Rheumatism (EULAR) Sjögren’s Syndrome Patients Reported Index; OHIP-14 – Oral Health Impact Profile – 14; ERQ – Emotion Regulation Questionnaire.
*** $p < 0.05$; ** $p < 0.001$.**

Discussion

Assessment of patients’ HRQoL is fundamental for thoroughly comprehending the level of the burden that the disease puts on their daily activities and well-being. The most significant areas of QoL related to health are physical, emotional, and social functioning. The PSS-QoL is the first disease-specific questionnaire for primary SS. It covers all aspects relevant to patients’ HRQoL, unlike general tools, and much more precisely than commonly used instruments, such as EQ-5D and ESSPRI.

The aim of the study was to translate the PSS-QoL questionnaire from English to Serbian, evaluate its psychometric properties, and validate it for use in the Serbian population. Preliminary results of this pilot research showed that the Serbian translation exhibits satisfactory feasibility, reliability, and validity.

The assessment of PSS-QoL’s reliability (Cα = 0.922) revealed similar results to the original questionnaire (Cα = 0.892) and HRQoL instruments for other rheumatic diseases, such as Rheumatoid QoL (RAQoL, Cα = 0.92) and Ankylosing Spondylitis QoL Questionnaire (ASQoL, Cα = 0.91) [9, 20, 21]. Likewise, the Serbian version of the PSS-QoL instrument demonstrated satisfactory temporal stability.

PCA identified only one factor that accounted for nearly 60% of the variance. Seeing that all the items had high values of factor weights, it could be concluded that they all measure the construct they are designed to, which is the psychosocial dimension of the HRQoL. Since the authors of the original study did not present their data regarding factor analysis, we could not compare our findings.

So far, the HRQoL of primary SS patients has been evaluated using generic measures, like EQ-5D. Our study demonstrated a moderate correlation between these two instruments. EQ-5D does not include some of the most important aspects of primary SS, especially dryness-related symptoms that might pose a great burden to patients’ daily lives [9, 22]. Therefore, the implementation of disease-specific questionnaires, like PSS-QoL, into routine clinical practice could lead to significant improvement in patient’s well-being as all of their needs would be addressed.

The results of our research indicated a strong correlation between PSS-QoL and ESSPRI domains. Both instruments emphasize that the sicca complex is an important predictor of altered HRQoL in pSS patients [22]. In the ESSPRI, dryness is evaluated as an overall item, while PSS-QoL provides a more precise description of all patient’s symptoms, not only regarding severity levels, and it also includes the psychosocial component of HRQoL.

OHIP-14 is an instrument designed to assess the impact of oral conditions, including xerostomia, on HRQoL domains [16]. Our study found that total scores of PSS-QoL and OHIP-14 correlate strongly. OHIP-14 psychological disability and PSS-QoL psychological dimension have a strong correlation as the social component is a very significant part of HRQoL, and it may be affected due to oral health problems. Xerostomia, a hallmark of primary SS, might lead to various intraoral manifestations and severe oral dysfunction [23]. The PSS-QoL questionnaire covers different oral-related symptoms, such as taste alteration, burning sensation, and dental problems. Therefore, it can be used for determining the extent of the oral disorders present during primary SS and their impact on HRQoL.

The main limitation of our study is the small sample size. Furthermore, we did not test and compare the results of different modes of questionnaire administration. In addition, one of the properties we could not evaluate is the questionnaire’s sensitivity to change, mostly related to the administration of effective medication, which is not yet widely available for patients with primary SS. Our next aim is to conduct prospective research involving more participants to re-assess the questionnaire’s psychometric properties in a more diverse group of primary SS patients regarding their sociodemographic characteristics, disease activity, and disease-related complications.

Conclusion

Our pilot study demonstrated that the Serbian version of the PSS-QoL questionnaire is as reliable as the original instrument in English. Therefore, the Serbian adaptation of the PSS-QoL instrument may be used to evaluate the HRQoL of patients with primary SS, both in academic research and everyday clinical practice, as a novel outcome measure. Still, future clinical trials that will include a more significant number of patients are needed to confirm further its validity in the Serbian population.

Conflict of interest

The authors declare no conflict of interest.

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The advantage of the platelet-to-lymphocyte ratio over neutrophil-to-lymphocyte ratio as novel markers of erythropoietin resistance in hemodialysis patients

Prednost odnosa trombocita i limfocita nad odnosom neutrofila i limfocita kao novih markera rezistencije na eritropoetin kod bolesnika na hemodijalizi

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Abstract

Background/Aim. Inflammation is one of the common factors that contribute to erythropoiesis stimulating agents (ESA) treatment resistance in hemodialysis patients. Lately, it is assessed by using new markers of inflammation, which are platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR). Their association with this therapy has not been fully investigated. The aim of the study was to evaluate the relationship between PLR, NLR, and ESA hyporesponsiveness index (EHRI). **Methods.** The research was conducted as a cross-sectional study and included 90 hemodialysis patients, who underwent clinical and laboratory testing in the form of physical examination and biochemical analyses. In all patients, the EHRI calculation was performed. **Results.** It is shown that EHRI had a statistically significant positive correlation with PLR ($p < 0.01$) and a negative correlation with hemoglobin levels ($p < 0.01$). Significant differences for logarithmically converted values of EHRI and PLR ($p < 0.05$) were found but not for EHRI and NLR ($p = 0.13$). **Conclusion.** Research has shown that PLR, together with NLR, could be used in assessing not only inflammation but also erythropoietin resistance in hemodialysis patients.

Key words:
blood platelets; erythropoietin; inflammation; lymphocytes; neutrophils; renal dialysis.

Apstrakt

Uvod/Cilj. Inflamacija je jedan od najčešćih faktora koji doprinose rezistenciji na terapiju agensima koji stimulišu eritropoezu (ASE) kod bolesnika na hemodijalizi. U novije vreme se procenjuje korišćenje novih markera upale, a to su odnos trombocita i limfocita (TLO) i odnos neutrofila i limfocita (NLO). Njihova povezanost sa terapijom eritropoetinom nije u potpunosti istražena. Cilj rada bio je da se proceni povezanost između TLO, NLO i ASE indeksa hiporesponsivnosti (AIHR). **Metode.** Istraživanje je sprovedeno kao studija preseka i obuhvatila je 90 bolesnika na hemodijalizi, kojima je urađeno kliničko i laboratorijsko ispitivanje u vidu fizikalnog pregleda i biohemijskih analiza. Kod svih bolesnika izračunat je AIHR. **Rezultati.** Utvrđeno je da je AIHR imao statistički značajnu pozitivnu korelaciju sa TLO ($p < 0,01$) i negativnu korelaciju sa nivoima hemoglobina ($p < 0,01$). Pronađene su značajne razlike za logaritamski konvertovane vrednosti AIHR i TLO ($p < 0,05$), ali ne i za IHRE i NLO ($p = 0,13$). **Zaključak.** Istraživanje je pokazalo da bi se TLO, zajedno sa NLO, mogao koristiti za procenu ne samo inflamacije, već i rezistencije na terapiju eritropoetinom kod bolesnika na hemodijalizi.

Ključne reči:
trombociti; eritropoetin; zapaljenje; limfociti; neutrofili; hemodijaliza.

Introduction

End-stage renal disease (ESRD) is characterized by many accompanying complications, out of which anemia is the most common and is associated with an increased risk of hospitalization and death. Inflammation is one of the key factors that contribute to erythropoiesis stimulating agents (ESA) hyporesponsiveness, and it is associated with increased activation of neutrophils and platelets (PLT). PLT-to-lymphocyte ratio (PLR) has been identified as a possible

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predictor of ESA hyporesponsiveness index (EHRI) and inflammation in ESRD [1].

It is unclear whether neutrophil-to-lymphocyte ratio (NLR) and/or PLR, which have been identified as markers of increased inflammation in ESRD patients, could have a relationship and clinical utility in assessing response to ESA and EHRI.

Almost 90% of all ESRD patients have anemia, and thus a diminished quality of life, and are at an increased risk of developing cardiovascular problems, leading to frequent hospitalization [2, 3].

Many causes of anemia in ESRD, such as inadequate dialysis, hyperparathyroidism, iron deficiency, occult blood loss, vitamin B12 deficiency, folate deficiency, have already been identified; however, the greatest contributor to anemia is the relative deficiency of erythropoietin (EPO) secretion from the affected kidney that is in correlation with the degree of anemia [4]. With the development and advent of ESA, a significant improvement in the treatment of anemia and its side effects has been achieved, resulting in a reduced need for blood transfusions and a lower mortality rate. Therefore, ESA therapy is nowadays the gold standard for the treatment of anemia in chronic kidney disease [5]. Even with this therapeutic approach, some patients have a reduced response to ESA therapy, which is defined as ESA hyporesponsiveness, with half (50%) of them being intermittent hyporesponders [6]. ESA hyporesponsiveness is defined as a failure to achieve the recommended target HGB levels despite a higher than usual dose of ESA or a continuous need for high doses to maintain the target HGB levels [7]. A meta-analysis conducted by Wish [6] describes ESA hyporesponsiveness as the failure to achieve the target HGB concentration of > 11 g/dL in patients who receive ESA dose equivalent to more than 500 IU/kg EPO per week or who have a prolonged need for such high dosages to maintain the target. Since HGB levels were not included by the aforementioned author, EHRI has been established and calculated as the weekly dose of ESA *per* kilogram of body weight divided by the HGB level (g/dL), making it extremely useful and easy to assess EPO resistance [8, 9].

One study has clearly identified many factors which have an impact on ESA responsiveness, among which inflammation, malnutrition, iron deficiency, secondary hyperparathyroidism, and inadequate dialysis are the most common and contribute to higher morbidity and mortality rates [10]. Furthermore, it is now widely known that patients with ESA hyporesponsiveness have increased inflammatory markers, which only indicates that inflammation is one of the main factors influencing the overall response [1].

In everyday common practice, many diagnostic and monitoring inflammatory markers are being used; however, new biological markers of inflammation are emerging. PLR and NLR have begun to be used not only in assessing cardiovascular risk and mortality but also in kidney patients and are tightly linked to inflammation and endothelial damage [1, 11]. Leukocyte count is strongly associated with increased cardiovascular mortality, and some studies find that certain subsets of leukocytes have even higher predictive value in the overall mortality, inflammation, and tissue damage than total white blood count. When NLR is used, such risk is even higher [12, 13].

In hemodialysis and peritoneal dialysis patients, NLR and PLR are associated with increased inflammation, whereas in the last couple of years, PLR was found to be associated with inflammation even more than NLR in these patients [11].

Taking into consideration the data mentioned above, the aim of the study was to identify NLR and/or PLR as markers of increased inflammation in ESRD patients and its relation and clinical utility in assessing ESA responsiveness and EHRI.

Methods

Study design and ethics statement

The study design was cross-sectional and it was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad (No. 01-39/28/1, from April 18, 2018). Written informed consent was obtained from all patients before enrollment. Inclusion criteria for enrollment in the study were the following: clinically stable patients older than 18 years of age, undergoing hemodialysis at the Dialysis Unit of Clinic for Nephrology and Clinical Immunology, University Clinical Center of Vojvodina, Serbia for at least six months, and receiving ESA treatment.

Study population and data collection

The study initially included 118 patients undergoing regular hemodialysis at our Unit. Those patients with a history of iron deficiency (serum ferritin values < 30 ng/mL), current infection, hematologic disorders and malignancies, history of blood transfusions and hospital admission in the last three months, or undergoing steroid treatment were excluded from the study, including patients with elevated C-reactive protein (CRP) levels in order to evaluate the relationship between EHRI, NLR, and PLR exclusively. In total, 28 patients were excluded from the study. After a thorough evaluation, 18 patients were excluded from the study. They had at least one of the exclusion criteria, i.e., mostly active infection, hematologic malignancies, and a history of recent blood transfusions. Ten patients did not receive ESA therapy due to satisfactory HGB levels (thus, no correction was required according to the guidelines; HGB level above 110 g/L) [6]. Demographic characteristics such as age, gender, body mass index (BMI), fat and lean tissue index, smoking status, dietary habits, etiology of ESRD, and medical history were recorded. In all patients, the dialysis prescription was three times a week for 4–5 hrs with blood flow rates of 300–400 mL/min, using a standard bicarbonate solution. A clinical examination was performed, with an emphasis on BMI and blood pressure. We collected three consecutive monthly laboratory records, which included blood count, renal function tests (blood urea, creatinine), data on mineral bone disease (calcium, phosphorus, intact parathormone), serum albumin, iron status (iron, transferrin, ferritin), and lipid status parameters (total cholesterol, triglycerides). The EHRI was calculated as the weekly ESA dose *per* kilogram of body weight divided by the HGB level (g/L). The mean HGB level

and EPO dose *per* month during three months were used for this calculation. The calculation of NLR was done by dividing the absolute neutrophil count (ANC) by the absolute lymphocyte count (ALC), whereas PLR was calculated by dividing the absolute PLT count by the ALC. Mean reference values and corresponding 95% reference intervals for the inflammatory markers, according to the study conducted on 8,711 healthy participants, for the NLR were 1.76 (0.83–3.92), and for PLR 120 (61–239) [14]. A study performed by Rabea et al. [7] showed that patients on hemodialysis who show signs of ESA hyporesponsiveness measured by EHRI have higher levels of PLR, with a median of 119.2 as opposed to patients in the group of good responsiveness who have lower levels of PLR with a median of 95.8.

Statistical analysis

The SPSS 20.0 software package was used for data processing (SPSS, Evanston, IL, USA). Descriptive statistics methods were used to measure central tendency (arithmetic mean, median) and measures of variability (standard deviation) in order to summarize the major numerical characteristics of observations. Data were checked for normality. The normality of the variables was analyzed using the Shapiro-Wilk test, which did not find evidence of a lack of normality in the residuals ($p = 0.20$). The Pearson coefficient was used for correlations. For the comparison of PLR among EHRI percentiles, the Kruskal-Wallis test was used. In the applied analyses, statistically significant differences were at the significance level of 95% ($p < 0.05$).

Results

Patient characteristics

The study was conducted on 90 patients undergoing maintenance dialysis and receiving ESA therapy. The median age of patients was 60.45 [± standard deviation (SD) 11.58] years, and, in total, 33 (36.7%) patients received darbepoetin alfa, whereas the remaining 57 patients received other short-acting ESA agents. The average HGB value in our sample was 105.79 (± SD 12.44) g/L, whereas the median applied dose of darbepoetin alfa *per* kilogram of body weight was 0.22 (± SD 0.14) mcg/kg and of short-acting ESA was 50.11 (± SD 28.11) IU/kg. In this study, we did not include patients with CRP levels to assess the relationship between NLR, PLR, and EHRI exclusively. Other descriptive data, demographic, clinical, and laboratory values are presented in Table 1.

Characteristics of EHRI and correlated novel markers of inflammation

In order to assess the correlation between EHRI and the other observed parameters, the Pearson correlation coefficient was used. In Table 2, EHRI values in all three meas-

Table 1

Descriptive data of the sample

Parameter	Reference range	N	Values
Age (years)		90	60.45 ± 11.58
EHRI[#]		81	4.98 (0.73–11.34)
Erythrocytes ($\times 10^{12}$/L)	3.9–5.4	90	3.43 ± 0.43
Hemoglobin (g/L)	120–160	81	105.79 ± 12.44
Hematocrit (%)	0.4–0.5	90	0.33 ± 0.037
CRP* (mg/dL)	0–5	87	5.20 (0.1–25.20)
Albumin (g/L)	35–52	87	36.93 ± 3.16
Ferritin (μg/L)	10–120	87	661.13 ± 484.39
Saturation transferrin (%)	15–50	87	30.12 ± 17.20

EHRI – ESA hyporesponsiveness index; ESA – erythropoiesis stimulating agents; CRP – C-reactive protein; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; N – number of patients.
All values are expressed as mean ± standard deviation except EHRI and CRP, which are expressed as mean (minimum–maximum).
Note: #EHRI was calculated by dividing weekly ESA dose per kilogram of body weight (IU/kg/week) by hemoglobin level (g/dL); *Three patients were not included in the study due to elevated CRP levels, in order to assess the relationship between NLR, PLR, and EHRI exclusively.

Table 2

Correlation between log EHRI and different independent variables measured in each of the three months

Parameter	Erythropoietin resistance		
	1st month	2nd month	3rd month
NLR	0.13	0.15	0.19
PLR	0.28**	0.30**	0.49**
HGB	-0.49**	-0.57**	-0.40**

EHRI – ESA hyporesponsiveness index; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; HGB – hemoglobin.
****$p < 0.01$.**

urements are presented. It is shown that EHRI had a statistically significant correlation with PLR of low to medium intensity (r_1 = 0.28, p < 0.01; r_2 = 0.30, p < 0.01; r_3 = 0.49, p < 0.01). There was a negative correlation of medium degree between EHRI and HGB levels (r_1 = -0.49, p < 0.01; r_2 = -0.57, p < 0.01; r_3 = -0.40, p < 0.01).

The correlation between NLR and PLR in all three measurements is shown in Table 3.

The relationship between the NLR, PLR, and the logarithmically converted levels of EHRI was examined firstly by using the Kruskal-Wallis test, which showed statistically significant differences for EHRI and PLR (p < 0.05) but not for EHRI and NLR (p = 0.13) in any of the three measurements. Significant differences in the distribution of EHRI for the different EHRI percentiles (p < 0.05) are presented in Figure 1. PLR from the first measurement (Figure 1A) was in correlation with logarithmically converted EHRI from the same measurement (p < 0.01). The Tukey multiple comparison tests were used to determine among which percentiles the PLR differences were found. *Post hoc* analysis indicated a statistically significant correlation between EHRI and PLR up to the 50th percentile (p < 0.05), while above the 50th percentile, there is no statistically significant correlation between EHRI and PLR. In the second measurement (Figure 1B), EHRI was associated with logarithmically converted EHRI (p < 0.01). *Post hoc* analysis revealed a statistically significant correlation between the 25th and 50th percentile (< 0.05) as well as for the 50th and 75th percentile (p < 0.05). PLR from the third measurement (Figure 1C) was associated with logarithmically converted EHRI from the third measurement (p < 0.01). Examination of *post hoc* analysis showed a significant correlation for the first (p < 0.05) and the second half of the curve (p < 0.01), bearing in mind that there was a stronger correlation between EHRI and PLR above the 50th percentile.

Discussion

In this study, we aimed to identify the correlation between EPO resistance and novel markers of inflammation NLR and PLR. To assess the response to EPO treatment, we used a body weight-adjusted dose of EPO and HGB levels as it is predefined by the EHRI equation. From the obtained results, we managed to demonstrate that PLR was independently associated with EHRI as opposed to NLR and EHRI. The results are consistent in all three measurements. These results are in agreement with other studies, while a possible explanation for these findings might lay in the fact that PLR is a better inflammatory marker than NLR [15–18]. Chávez et al. [19] found that the correlation between PLR and inflammatory parameters was superior to that obtained by NLR. Opposite to our research, Valga et al. [18] found a significant association of the EHRI with both parameters, whereas low lymphocyte levels were given as a possible explanation for this finding.

Many factors have already been independently associated with ESA resistance; however, inflammation is still the leading cause of this condition [19]. Chronic inflammation, identified most commonly by elevated levels of CRP, interleukin (IL)-6, and tumor necrosis factor (TNF)-alpha, presents a part of malnutrition-inflammation-atherosclerosis syndrome in patients with ESRD [19–22]. Novel inflammation markers PLR and NLR have been found to be in positive correlation with IL-6 and TNF-α so much that PLR was better than NLR at predicting inflammation [16]. Okyay et al. [23] demonstrated a strong positive correlation between NLR and inflammatory cytokines such as IL-6 and CRP.

PLT have a wide range of interactions with different cell subsets, and there is emerging evidence that they can influence leukocyte recruitment, causing inflammation, which is the pathogenic mechanism of atherosclerosis, making them an important factor in this process by actively secreting pro-

Table 3

Correlation between NLR and PLR in all three measurements

Parameter	NLR		
	r_1	r_2	r_3
NLR	-	-	-
PLR	0.63**	0.71**	0.53**

For abbreviations see Table 2. **p < 0.01.

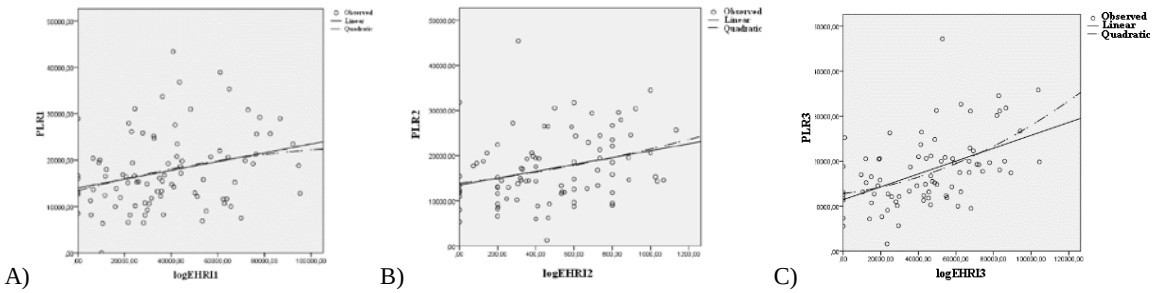

Fig. 1 – Correlation between PLR and logarithmically converted EHRI in three measurements. For abbreviations see Table 2.

inflammatory cytokines [23–26]. The advantage of the PLR is that it shows both hyperactive coagulation and inflammatory pathways, making it superior to the individual PLT or lymphocyte counts in the prediction of inflammation and consequential EPO resistance [26].

In our research, 10% of patients had HGB levels over 110 g/L; therefore, in accordance with international guidelines, no EPO treatment was administered [7]. In the remaining patients, HGB levels were corrected by an individually adjusted ESA dosing regimen. In this research, we have found a statistically significant negative correlation between the HGB levels and therapeutic response to ESA represented by EHRI, which indicates that less stable levels of HGB have a negative impact on ESA treatment response.

The obtained results show a positive association between NLR and PLR, which indicates that in inflammatory conditions, there is simultaneous activation of these blood cells, and they might be considered predictors of ESA resistance in ESRD patients. In patients undergoing maintenance dialysis with an ongoing inflammatory process, PLR and NLR are elevated [18, 26]. Meanwhile, inflammation in hemodialysis patients is a risk factor contributing to higher morbidity and mortality rates [9]. Additionally, a statistically significant difference was found between groups of patients with resistance to EPO and PLR once divided into percentile ranges. The data obtained from our research only confirms previous findings that show that PLR could be a good marker of EHRI and possibly inflammation in the population of patients with stage V of chronic kidney disease [10].

Conclusion

By being both financial and time-consuming, PLR and NLR are universally accessible methods and could be used in screening and evaluation of inflammation in ESRD patients, thereby assessing ESA response. Simple calculations of PLR are superior to NLR in predicting ESA response in these patients. We firmly believe that these markers, especially PLR, can be routinely used in everyday dialysis practice as markers of not only mortality but also inflammation and therapeutic response. However, additional randomized and controlled studies are imperative to assess PLR, NLR, and EHRI in ESRD patients.

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Quality of life after maxillectomy and prosthetic rehabilitation – a pilot study

Kvalitet života nakon maksilektomije i protetske rehabilitacije: pilot studija

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Abstract

Background/Aim. Although radical surgical treatment is the method of choice in the therapy of cancer of the maxilla and maxillary sinus, it can cause oral dysfunction, social isolation, and emotional and psychological distress, which significantly affects the patient's quality of life (QoL). The aim of the study was to determine the health-related QoL of patients rehabilitated with obturator prosthesis (OP) after maxillectomy, according to demographic and clinical characteristics. **Methods.** The study included 32 patients with a mean age of 63.6 years. The measurement of QoL of patients after maxillectomy and prosthetic rehabilitation was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTC QLQ-C30) and EORTC QLQ – Head and Neck Cancer Module (QLQ-H&N43). **Results.** Functioning and symptoms were more pronounced in women, younger patients, and single patients ($p < 0.05$). Patients after definitive prosthetics rehabilitation had fewer problems and symptoms compared to patients with surgical and interim OP ($p < 0.05$). Patients with partial obturator dentures had a better perception of function than patients with total dentures ($p < 0.05$). Irradiated patients had more pronounced fatigue, appetite loss, pain in the mouth, dry mouth and sticky saliva, and sense and skin problems compared to nonirradiated patients ($p < 0.05$). **Conclusion.** The results of this study suggest that sex, age, marital status, characteristics of OP, and radiotherapy have a significant impact on QoL in patients after maxillectomy.

Key words:
head and neck neoplasms; maxillofacial prosthesis; oral surgery procedures; quality of life; rehabilitation; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Iako je radikalno hirurško lečenje metod izbora u lečenju tumora gornje vilice i maksilarnih sinusa, ono može dovesti do oralnih disfunkcija, socijalne izolacije, kao i emocionalnog i psihološkog stresa, što značajno utiče na kvalitet života (KŽ) bolesnika. Cilj rada bio je da se ispita KŽ povezan sa zdravljem bolesnika kod kojih je posle maksilektomije primenjena opturator proteza (OP), u odnosu na njihove demografske i kliničke karakteristike. **Metode.** Istraživanjem su obuhvaćena 32 bolesnika, prosečne starosti 63,6 godina. Merenje KŽ bolesnika posle maksilektomije i protetske rehabilitacije vršeno je korišćenjem *European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30* (EORTC QLQ-C30) upitnika i posebnim modulom upitnika za tumore glave i vrata – EORTC QLQ – *Head and Neck Cancer Module* (QLQ-H&N43). **Rezultati.** Poremećaji funkcionisanja i simptomi bili su značajno izraženiji kod žena, mlađih bolesnika i samaca ($p < 0,05$). Nakon rehabilitacije definitivnom opturator protezom (OP), bolesnici su imali manje problema i simptoma u odnosu na bolesnike sa hirurškom ili *interim* OP ($p < 0,05$). Bolesnici sa parcijalnim OP imali su bolju percepciju funkcije u odnosu na bolesnike sa totalnim protezama ($p < 0,05$). Zračeni bolesnici imali su izraženiji umor, gubitak apetita, bol u ustima, suva usta i lepljivu pljuvačku, probleme sa čulima i kožom, u poređenju sa nezračenim bolesnicima ($p < 0,05$). **Zaključak.** Rezultati ovog istraživanja ukazuju na to da pol, starost, bračni status, karakteristike OP i radioterapija imaju značajan uticaj na KŽ bolesnika nakon maksilektomije.

Ključne reči:
glava i vrat, neoplazme; proteze, maksilofacijalne; hirurgija, oralna, procedure; kvalitet života; rehabilitacija; ankete i upitnici.

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Introduction

Head and neck cancer (HNC) accounts for approximately 900,000 cases and over 400,000 deaths annually worldwide [1]. According to the latest available data, in the general population of Serbia, the incidence rate of oral cancer was 6.5/100,000 and from maxillary sinus 0.7/100,000 [2].

The radical surgical procedure is the most frequent treatment for cancer of the maxilla and maxillary sinus. Postsurgical maxillectomy defects can cause oral dysfunction, social isolation, and emotional and psychological distress, which may altogether significantly affect the patient's quality of life (QoL) [3]. Patients need to be able to return to a normal life after maxillectomy without functional impairment or psychological trauma due to aesthetical disfigurement.

Dental rehabilitation is one of the major steps towards the improvement of the QoL after extensive surgical procedures [4]. The use of a prosthetic obturator enables closure of the maxillary defect, separates the oral cavity from the sinonasal cavities, and thus avoids regurgitation [5]. A successful obturator prosthesis (OP) improves speech, mastication, swallowing, and esthetics, which significantly improves the overall well-being of the patient [6–9].

One of the most important parameters in examining the effects of post-treatment follow-up in HNC patients is health-related QoL. Various cross-sectional studies have evaluated the patient's QoL after rehabilitation with OP by using specific questionnaires [4, 10–12].

The most commonly used instruments to assess health-related QoL in patients with HNC are the 30-item Cancer-Quality of Life Questionnaire (QLQ) - QLQ-C30 [13] and 35-item Head and Neck Cancer-QLQ module (QLQ-H&N35) [14], developed by the European Organization for Research and Treatment of Cancer (EORTC). The QLQ-H&N43 questionnaire is a revised and updated version of the QLQ-H&N35. The European Organization for Research and Treatment of Cancer QLQ Core 30 (EORTC QLQ-C30) and the module QLQ-H&N43 instruments have been translated into many languages, including Serbian, and are reliable and valid assessment tools of the QoL of patients with the HNC in multicultural clinical research [15, 16].

To date, the measurement of the QoL in patients with maxillectomy in Serbia has not been given adequate attention. To the best of our knowledge, this is the first study of its kind to investigate the QoL after maxillectomy and prosthetic rehabilitation in HNC patients in the population of Serbia.

The aim of the study was to determine the health-related QoL of patients rehabilitated with OP after maxillectomy according to demographic and clinical characteristics.

Methods

Study design and participants

This retrospective cross-sectional study included patients who underwent surgical maxillectomy and were rehabilitated with OP at the University of Belgrade, Faculty of Dental Medicine, Clinic for Maxillofacial Surgery, Serbia. The study was conducted from October to December 2019. The eligibility criteria of patients included the following: surgical maxillectomy, subsequent rehabilitation treatment with OP, and full completion of the self-reported questionnaire. Exclusion criteria included: recurrent disease, severe comorbidities, free flaps rehabilitation treatment, zygomaticus implant-retained prosthesis, composite occlusal resection (resection of mandible), severe trismus, and noncooperative behavior. Thirty-two patients who met the criteria were analyzed (Figure 1).

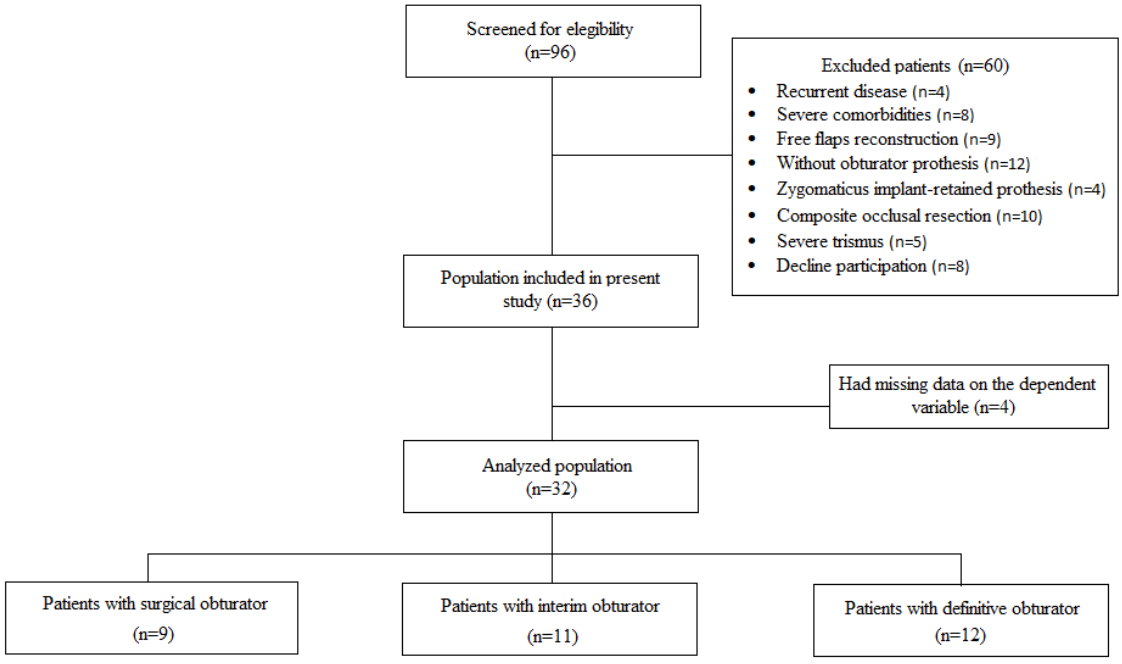

Fig. 1 – Flowchart of participants.

After maxillectomy, patients were rehabilitated with surgical, interim, or definitive OP. The process from surgical treatment to rehabilitation with different types of OP has been previously described by others [7]. For patients who underwent radiation therapy, after the improvement of the general condition, the moment of definitive prosthetic treatment was individually assessed. Assessment of QoL through a questionnaire was evaluated one week after the beginning of wearing and adjusting any type of obturator.

The study was approved by the Ethical Committee of the Faculty of Dental Medicine, University of Belgrade (No. 36/13) and conducted following the Declaration of Helsinki. All patients provided written informed consent to participate in this study.

Instruments

Patients' health-related QoL was assessed individually using EORTC QLQ-C30 (version 3.0) and QLQ-H&N43. The Serbian version was provided by the EORTC group.

The EORTC QLQ-C30 contains 30 questions and includes a single global health/QoL scale scored on a seven-point Likert scale, five functional scales (physical, role, emotional, cognitive, social), three symptom domains (fatigue, nausea/vomiting, pain), and six single items (dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties) scored on a Likert-like response format (1 – not at all; 2 – a little; 3 – quite a bit; 4 – very much) [16]. Scoring is done according to the EORTC scoring manual [17]. During the scoring procedure, raw EORTC QLQ-C30 scores are linearly transformed into 0–100 scales. In the QLQ-C30 questionnaire, for global health status and the five functioning scales, a high score corresponds to a high QoL. For a symptom scale/items, a higher score implies maximum difficulty or symptom burden.

The QLQ-H&N43 questionnaire consists of 43 questions about the symptoms and side effects of cancer treatment. The questionnaire includes 12-item symptom scales (pain, swallowing, speech problems, senses problems, social eating, problems with teeth, body image, skin problems, sexuality, dry mouth/sticky saliva, shoulder problems, and anxiety) and seven single-item symptom scales (social contact, opening mouth, coughing, lymphedema, weight loss, problems with wound healing, and neurological problems). All items have a four-point Likert scale. All of the multi-item scales and single-item measures range in score from 0 to 100 according to the EORTC scoring manual [15, 16]. In the QLQ-H&N43 questionnaire, a high score represents a high level of difficulties or problems.

A demographic questionnaire was used to collect information about sex, age, marital status, education level, employment, and economic status. Clinical parameters were taken from hospital records and included information about the tumor location, oncological treatment, type of surgical treatment, the time elapsed from maxillectomy to prosthetic rehabilitation, the type of prosthetic obturator reconstruction (surgical, interim, definitive), and the type of definitive OP (total and partial).

Patients were invited to participate in the study during a clinic visit in the order of appearance. The participants filled out questionnaires by themselves. It took 25–30 min on average for each participant to answer the questionnaires. All patients were treated by an experienced maxillofacial prosthodontist who rehabilitates patients with maxillary defects.

Statistical analysis

Several different methods were used to perform the statistical analysis: descriptive summary statistics for the demographic and clinical characteristics and EORTC QLQ-C30 and QLQ-H&N43 scores; parametric (*t*-test) and nonparametric statistic tests (χ^2 and Fisher exact test) for comparison analyses; nonparametric statistics (Mann-Whitney *U* test). A statistical significance was set at $p < 0.05$. Software package SPSS 22 was used for the analyses (SPSS inc, Chicago, IL, USA).

Results

Of the 96 patients with maxillectomy, the remaining 32 patients with surgical, interim, or definitive OP who met all criteria and completed the questionnaire entirely were analyzed.

Demographic and clinical parameters are presented in Table 1. The results have shown that the patients were predominantly in partnerships or marital unions, with completed secondary education, retired, and of average economic status. The primary localization of the tumor was the maxillary alveolus, hard palate, and maxillary sinus. Most of the patients were treated with surgery (predominantly with partial maxillectomy) and postoperative radiotherapy. The time elapsed from maxillectomy to prosthetic rehabilitation and the type of OP (total/partial) are presented in Table 1.

There was no statistically significant difference in QoL compared to education, employment, and economic status. The average score value of different scales for EORTC QLQ-C30 and QLQ-H&N43 according to sex, age, and marital status (single vs. partnership/marriage) is given in Table 2. The functioning scale with the lowest average score in both sexes was global health status. Women had significantly worse scores in emotional functioning and felt more pronounced symptoms such as fatigue, insomnia, and appetite loss ($p < 0.05$) ($p = 0.016$, $p = 0.027$, $p = 0.032$, respectively). The men were most affected in terms of social functioning, financial difficulties, and sexuality problems ($p < 0.05$) ($p = 0.045$, $p = 0.004$, $p = 0.024$, respectively).

Younger patients had higher values for global health status but without statistical significance. The emotional and social functioning in patients under 60 years of age was significantly lower than in patients over the age of 60 ($p < 0.05$) ($p = 0.038$ and $p = 0.034$, respectively). Dominant symptoms in the in patients over the age of 60 were speech problems, problems with social eating, sexuality, and social contact ($p < 0.05$) ($p = 0.022$, $p = 0.036$, $p = 0.016$, $p = 0.043$, respectively) (Table 2).

Table 1

Demographic and clinical characteristics

Characteristics	Patients (n = 32)
Gender	
male	19 (59.4)
female	13 (40.6)
Age (years)	63.6 (44–83)
Marital status	
married/partner	19 (59.4)
divorced	5 (15.6)
widowed	8 (25.0)
Education level	
no formal education	3 (9.4)
primary school	7 (21.8)
secondary school	17 (53.2)
university	5 (15.6)
Employment status	
employed	8 (25.0)
unemployed	4 (12.5)
retired	20 (62.5)
Economic status	
good	5 (15.6)
moderate	14 (43.8)
bad	10 (31.3)
very bad	3 (9.3)
Localization	
maxillary alveolus	13 (40.6)
hard palate	8 (25.0)
maxillary sinus/nasal cavity	11 (34.4)
Treatment	
surgery only	14 (43.8)
surgery + radiotherapy	12 (37.5)
surgery + chemotherapy	4 (12.4)
surgery + radiotherapy + chemotherapy	2 (6.3)
Surgical procedure	
partial maxillectomy	22 (68.8)
subtotal maxillectomy	4 (12.5)
total maxillectomy	6 (18.7)
Time elapsed from maxillectomy and prosthetic rehabilitation	
surgical obturator prostheses (days)	4.3 ± 2.2
interim obturator prostheses (weeks)	3.2 ± 1.3
definitive obturator prostheses	
nonirradiated patients (weeks)	8.5 ± 2.5
irradiated patients (months)	22.4 ± 10.7
Type of obturator prostheses	
total denture	14 (43.8)
partial denture	18 (56.2)

All results are expressed as numbers (percentages) except age and time elapsed from maxillectomy and prosthetic rehabilitation which are expressed as median (range) and mean ± standard deviation, respectively.

The global health status was slightly higher in patients who were married or in partnership but without statistical significance. The single patients had lower scores in all functioning scales, but the physical functioning was statistically significantly lower as compared to patients with the "in marriage/partnership" status ($p < 0.05$) ($p = 0.042$). Among the symptoms, fatigue and financial problems were significantly compromised in single patients than in those living in a marital or partnership union ($p < 0.05$) ($p = 0.025$ and $p = 0.027$, respectively) (Table 2).

The average score value of different scales for EORTC QLQ-C30 and QLQ-H&N43 according to the type of OP, denture, and treatment are given in Table 3. Regardless of the type of prosthesis, the functioning scale with the lowest average score was global health status, while functioning scales with the highest scores were observed in the cognitive, physical, and role domains. The highest value for global health status was in patients with definitive obturators compared to patients with surgical and interim obturators but without statistical significance. Significant differences were observed in the fatigue, insomnia, constipation, appetite loss, dry mouth, speech problems, and social eating domains in patients with surgical OPs compared to patients with definitive OP ($p < 0.05$) ($p = 0.002$, $p = 0.028$, $p = 0.021$,

Table 2

The average score value of EORTC QLQ-C30 and QLQ-H&N43 according to age, sex, and living arrangements

Scale/items	Gender		*p*	Age (years)		*p*	Marital status		*p*
	male	female		< 60	> 60		single	marriage/ partnership	
EORTC QLQ-C30									
global health status	53.2 ± 17.3	48.7 ± 23.7	0.209	54.4 ± 21.2	45.6 ± 25.3	0.426	47.5 ± 23.6	51.2 ± 17.3	0.309
physical functioning	80.3 ± 30.4	73.8 ± 25.9	0.221	73.5 ± 18.4	67.3 ± 27.5	0.432	77.8 ± 26.5	90.1 ± 34.3	0.042*
role functioning	81.5 ± 24.3	76.7 ± 21.1	0.584	70.4 ± 27.9	77.9 ± 30.7	0.235	70.1 ± 28.4	83.8 ± 29.9	0.884
emotional functioning	68.3 ± 23.4	52.3 ± 24.3	0.037*	62.8 ± 29.6	80.9 ± 21.9	0.038*	58.7 ± 18.6	62.3 ± 23.5	0.056
cognitive functioning	74.2 ± 19.9	77.4 ± 18.5	0.869	70.9 ± 21.6	68.1 ± 22.7	0.863	64.6 ± 25.7	67.9 ± 27.3	0.534
social functioning	62.2 ± 27.3	75.3 ± 26.2	0.045*	66.3 ± 29.8	77.4 ± 26.6	0.034*	61.9 ± 19.8	75.4 ± 30.8	0.654
fatigue	27.2 ± 10.8	42.1 ± 17.3	0.016*	24.5 ± 21.6	29.3 ± 28.6	0.842	41.4 ± 15.8	20.3 ± 2.7	0.025*
nausea and vomiting	7.5 ± 7.2	5.4 ± 2.3	0.653	2.1 ± 2.0	3.5 ± 3.3	0.342	6.3 ± 5.3	8.1 ± 7.5	0.520
pain	22.1 ± 17.5	13.2 ± 11.2	0.368	13.7 ± 11.3	20.9 ± 17.8	0.345	21.7 ± 15.7	27.4 ± 17.9	0.169
dyspnea	11.8 ± 9.7	3.5 ± 3.3	0.126	6.9 ± 5.8	3.8 ± 3.3	0.431	4.5 ± 3.3	6.3 ± 5.2	0.828
insomnia	14.7 ± 12.1	33.3 ± 10.5	0.027*	28.1 ± 17.3	23.6 ± 10.4	0.452	21.6 ± 10.7	22.4 ± 21.6	0.279
appetite loss	16.7 ± 22.4	32.6 ± 21.7	0.032*	33.2 ± 15.6	20.9 ± 17.5	0.356	24.8 ± 21.3	13.3 ± 9.9	0.519
constipation	9.8 ± 7.6	16.0 ± 7.3	0.350	14.3 ± 13.6	7.5 ± 6.9	0.534	5.4 ± 4.5	3.9 ± 2.3	0.851
diarrhea	9.3 ± 5.3	11.1 ± 10.1	0.605	13.6 ± 10.5	7.2 ± 6.4	0.438	16.8 ± 5.5	17.6 ± 11.5	0.719
financial difficulties	47.9 ± 26.3	20.4 ± 19.6	0.004*	30.7 ± 10.7	36.4 ± 29.3	0.137	44.9 ± 19.1	23.4 ± 17.4	0.027*
EORTC QLQ-H&N43									
pain in the mouth	18.4 ± 21.0	28.4 ± 24.3	0.105	4.2 ± 5.9	13.9 ± 12.7	0.234	21.9 ± 20.1	18.3 ± 14.4	0.272
swallowing	22.7 ± 25.9	22.5 ± 19.3	0.978	16.7 ± 23.6	16.8 ± 15.7	0.395	23.7 ± 22.6	16.7 ± 28.9	0.346
problems with teeth	23.4 ± 26.6	33.3 ± 29.5	0.128	16.7 ± 23.6	3.7 ± 6.4	0.527	28.6 ± 26.8	19.1 ± 17.2	0.375
dry mouth and sticky saliva	23.6 ± 29.0	29.0 ± 29.4	0.489	15.0 ± 35.3	5.6 ± 9.6	0.382	21.9 ± 24.2	26.7 ± 16.7	0.436
senses problems	15.5 ± 24.8	16.0 ± 27.5	0.940	12.2 ± 10.3	22.1 ± 19.3	0.253	20.2 ± 31.7	27.8 ± 48.1	0.732
speech problems	20.0 ± 26.3	25.2 ± 21.9	0.429	43.2 ± 4.2	25.6 ± 13.4	0.022*	28.4 ± 26.5	25.1 ± .19.9	0.138
body image	26.4 ± 26.5	26.3 ± 24.7	0.988	33.3 ± 26.2	20.2 ± 38.5	0.052	36.8 ± 26.2	33.3 ± 48.4	0.680
social eating	25.3 ± 22.9	35.5 ± 29.1	0.148	37.5 ± 53.0	22.2 ± 21.0	0.036*	33.3 ± 32.2	29.7 ± 14.4	0.159
sexuality	45.9 ± 27.4	28.6 ± 18.2	0.024*	41.7 ± 16.7	16.7 ± 16.7	0.016*	37.4 ± 16.6	43.6 ± 41.9	0.529
shoulder problems	6.3 ± 16.9	11.1 ± 18.5	0.316	18.0 ± 19.4	5.6 ± 9.6	0.373	14.0 ± 19.5	18.4 ± 17.6	0.503
skin problem	6.9 ± 15.5	9.9 ± 14.6	0.463	5.6 ± 7.9	8.3 ± 9.5	0.314	8.7 ± 5.7	3.7 ± 2.4	0.516
anxiety	45.9 ± 32.0	43.5 ± 32.4	0.136	25.7 ± 17.1	22.2 ± 19.5	0.641	43.0 ± 35.7	44.4 ± 38.5	0.149
problems opening mouth	16.1 ± 26.2	25.9 ± 32.5	0.216	28.3 ± 28.6	32.4 ± 24.3	0.531	26.3 ± 30.6	18.1 ± 19.2	0.452
coughing	14.9 ± 21.1	17.3 ± 26.7	0.716	16.7 ± 23.6	15.1 ± 14.1	0.825	14.0 ± 23.1	17.0 ± 13.2	0.861
social contact	33.3 ± 36.7	48.1 ± 32.5	0.117	36.7 ± 23.6	22.2 ± 18.2	0.043*	40.3 ± 30.6	45.6 ± 28.9	0.487
swelling in the neck	1.1 ± 6.2	6.2 ± 16.1	0.124	9.7 ± 9.2	13.7 ± 10.2	0.323	3.5 ± 2.3	6.1 ± 5.2	0.328
weight loss	27.6 ± 25.3	19.7 ± 24.9	0.249	37.0 ± 23.6	29.3 ± 18.3	0.314	23.6 ± 14.4	22.2 ± 19.2	0.156
problem with wound healing	2.3 ± 12.4	7.4 ± 19.3	0.239	7.6 ± 5.3	8.5 ± 6.8	0.682	10.5 ± 22.4	12.2 ± 13.3	0.887
neurological problems	5.7 ± 18.0	11.1 ± 25.0	0.123	8.2 ± 6.3	9.3 ± 5.6	0.538	14.0 ± 11.6	11.7 ± 10.4	0.571

EORTC QLQ-C30 – European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ) Core 30; QLQ-H&N43 – EORTC QLQ Head and Neck Module.
All results are expressed as mean ± standard deviation. *$p < 0.05$.

$p = 0.048$, $p = 0.042$, $p = 0.013$, $p = 0.035$, respectively). Further, a significant difference between QoL with surgical OPs and QoL with interim OPs was found only in constipation ($p < 0.05$) ($p = 0.034$). A significant difference between QoL with interim OP and QoL with definitive OPs was found in speech problems, social eating, skin problems, and social contact domain ($p < 0.05$) ($p = 0.013$, $p = 0.035$, $p = 0.032$, $p = 0.016$, respectively).

In case all maxillary teeth were missing, the OP was made with all teeth, while in the presence of the remaining teeth, the denture would compensate for the missing teeth. Patients with definitive total denture had more pronounced symptoms such as problems with swallowing, senses, speech, social eating, and social contact compared to patients with a partial denture ($p < 0.05$) ($p = 0.026$, $p = 0.016$, $p = 0.037$, $p = 0.041$, $p = 0.043$, respectively).

In relation to using radiation therapy, patients in whom radiotherapy was used had a worse perception of global health status and all functional scales but without statistical significance. Irradiated patients had more pronounced symptoms such as fatigue, appetite loss, pain in the mouth, dry mouth and sticky saliva, and sense and skin problems compared to nonirradiated patients ($p < 0.05$) ($p = 0.014$, $p = 0.002$, $p = 0.018$, $p = 0.010$, $p = 0.002$, $p = 0.001$, respectively).

Discussion

Postsurgical maxillary defects predispose the patient to have several functional, emotional, and social problems. Studies have shown that clinicians miss up to half of the self-reported subjective toxicities reported by patients with canc-

Table 3

The average score value of EORTC QLQ-C30 and QLQ-H&N43 according to the type of obturator prosthesis, denture, and treatment

Scale/items	Type of obturator prostheses								Treatment		
	surgical	interim	definitive	*p*	Sig. time interval	total denture	partial denture	*p*	irradiated	nonirradiated	*p*
EORTC QLQ-C30											
global health status	42.7 ± 19.5	52.6 ± 20.5	60.8 ± 18.2	0.092		52.3 ± 23.8	65.7 ± 29.7	0.318	56.8 ± 15.4	64.2 ± 20.5	0.124
physical functioning	64.3 ± 19.6	72.3 ± 18.7	76.6 ± 19.2	0.085		61.4 ± 25.2	75.5 ± 23.6	0.366	62.1 ± 18.5	77.3 ± 19.9	0.079
role functioning	68.8 ± 25.3	71.7 ± 22.5	77.9 ± 18.2	0.204		69.4 ± 29.4	72.9 ± 19.5	0.291	73.8 ± 26.3	79.8 ± 21.2	0.137
emotional functioning	61.9 ± 25.6	61.4 ± 25.2	65.7 ± 19.6	0.336		64.8 ± 26.8	66.4 ± 21.0	0.153	61.7 ± 22.6	68.9 ± 19.6	0.098
cognitive functioning	76.8 ± 13.6	77.6 ± 17.1	79.6 ± 19.8	0.447		71.3 ± 25.2	76.8 ± 24.6	0.239	76.7 ± 18.6	79.8 ± 23.7	0.328
social functioning	63.7 ± 25.6	67.2 ± 24.6	75.0 ± 19.7	0.135		72.8 ± 23.2	75.6 ± 22.6	0.241	73.7 ± 23.6	76.7 ± 22.3	0.268
fatigue	41.9 ± 25.7	31.8 ± 19.6	24.2 ± 26.5	0.002*	S/D	21.2 ± 21.7	26.5 ± 21.6	0.248	43.9 ± 22.1	27.1 ± 19.7	0.014*
nausea and vomiting	12.7 ± 19.2	9.7 ± 11.7	6.7 ± 10.4	0.114		12.7 ± 12.4	9.5 ± 5.3	0.138	14.7 ± 12.1	13.8 ± 10.2	0.257
pain	25.3 ± 26.1	17.8 ± 18.4	13.6 ± 15.8	0.097		13.8 ± 12.6	14.6 ± 13.6	0.362	24.3 ± 22.3	32.3 ± 26.1	0.168
dyspnea	13.1 ± 21.5	11.3 ± 19.5	6.9 ± 11.9	0.069		9.5 ± 7.1	9.2 ± 4.8	0.424	8.9 ± 15.5	6.1 ± 10.3	0.325
insomnia	30.0 ± 33.6	18.2 ± 24.7	8.9 ± 15.1	0.028*	S/D	19.1 ± 20.2	17.3 ± 14.6	0.162	10.0 ± 11.6	8.9 ± 15.1	0.174
appetite loss	32.9 ± 26.7	25.0 ± 26.1	14.5 ± 21.8	0.021*	S/D	12.2 ± 8.5	8.8 ± 9.4	0.247	16.9 ± 16.7	13.5 ± 21.7	0.002*
constipation	25.6 ± 32.4	12.7 ± 13.2	5.6 ± 8.4	0.034*	S/I, S/D 0.048	10.6 ± 6.5	7.6 ± 7.2	0.243	8.6 ± 9.4	6.6 ± 7.5	0.185
diarrhea	13.5 ± 17.8	8.2 ± 11.0	4.5 ± 8.4	0.057		9.4 ± 9.1	8.1 ± 5.3	0.384	6.3 ± 5.6	4.8 ± 5.4	0.236
financial difficulties	35.4 ± 39.8	42.5 ± 42.2	37.6 ± 39.1	0.859		35.6 ± 21.5	34.1 ± 30.1	0.162	38.8 ± 22.3	37.7 ± 35.1	0.291
EORTC QLQ-H&N43											
pain in the mouth	24.7 ± 29.8	19.8 ± 19.9	12.9 ± 13.2	0.069		19.2 ± 15.2	18.1 ± 14.2	0.536	21.8 ± 13.6	4.2 ± 5.8	0.018*
swallowing	34.1 ± 35.2	21.3 ± 25.3	25.0 ± 25.8	0.156		29.2 ± 12.9	17.0 ± 11.8	0.026*	19.7 ± 18.3	6.0 ± 5.7	0.131
problems with teeth	24.1 ± 19.9	22.5 ± 25.5	21.4 ± 23.9	0.679		23.4 ± 20.4	28.2 ± 21.6	0.186	20.9 ± 15.7	21.4 ± 23.2	0.252
dry mouth and sticky saliva	37.5 ± 39.6	26 ± 29.2	18.5 ± 19.6	0.042*	S/D	25.2 ± 20.3	22.5 ± 19.2	0.266	31.6 ± 28.6	12.7 ± 13.5	0.010*
senses problems	9.7 ± 13.1	12.5 ± 15.5	11.6 ± 13.5	0.944		21.5 ± 18.7	12.1 ± 10.4	0.016*	45.6 ± 23.5	13.3 ± 16.2	0.002*
speech problems	67.3 ± 25.6	51.1 ± 20.2	30.9 ± 17.6	0.013*	S/D, I/D 0.016	39.4 ± 21.5	29.9 ± 19.7	0.037*	33.1 ± 25.6	30.7 ± 18.5	0.337
body image	16.2 ± 15.9	19.4 ± 17.1	14.3 ± 20.2	0.203		17.3 ± 12.4	15.3 ± 9.5	0.472	25.0 ± 18.6	17.3 ± 19.1	0.168
social eating	42.2 ± 45.6	48.6 ± 42.5	21.1 ± 22.9	0.035*	S/D, I/D 0.026	38.7 ± 32.1	27.7 ± 29.5	0.041*	24.9 ± 26.8	20.1 ± 21.5	0.238
sexuality	17.6 ± 23.6	21.7 ± 29.8	10.6 ± 15.3	0.558		19.2 ± 18.4	19.8 ± 20.8	0.482	15.6 ± 12.4	10.7 ± 9.3	0.469
shoulder problems	8.7 ± 12.2	13.5 ± 16.0	12.6 ± 13.9	0.823		11.6 ± 10.7	12.2 ± 11.9	0.211	18.3 ± 18.1	13.6 ± 11.2	0.275
skin problem	29.5 ± 32.4	41.2 ± 22.3	18.5 ± 23.4	0.032*	I/D 0.039	17.5 ± 13.5	15.6 ± 10.4	0.325	43.8 ± 22.1	19.3 ± 20.2	0.001**
anxiety	37.6 ± 29.4	27.6 ± 30.9	22.6 ± 28.8	0.532		24.6 ± 18.6	25.0 ± 13.8	0.357	24.9 ± 22.4	20.6 ± 20.2	0.239
problems opening mouth	34.0 ± 28.4	38.7 ± 39.5	30.2 ± 17.8	0.568		28.2 ± 28.6	26.2 ± 25.3	0.423	33.8 ± 23.6	28.2 ± 23.4	0.362
coughing	23.2 ± 27.5	18.5 ± 21.1	9.3 ± 15.2	0.478		11.3 ± 10.2	10.5 ± 9.2	0.123	13.7 ± 6.3	8.9 ± 11.8	0.628
social contact	29.4 ± 25.8	32.9 ± 13.5	11.1 ± 10.5	0.016*	I/D	29.6 ± 30.6	17.6 ± 15.7	0.043*	17.9 ± 16.7	12.1 ± 10.2	0.357
swelling in the neck	10.4 ± 13.5	8.5 ± 10.4	8.4 ± 11.2	0.432		5.4 ± 4.3	5.6 ± 5.2	0.226	8.7 ± 6.3	8.9 ± 9.4	0.507
weight loss	67.2 ± 53.7	57.2 ± 51.3	35.3 ± 41.5	0.124		24.2 ± 23.1	20.6 ± 20.1	0.256	37.3 ± 28.6	32.3 ± 25.7	0.284
problems with wound healing	5.7 ± 3.4	4.1 ± 4.5	3.2 ± 3.6	0.342		3.0 ± 2.6	4.2 ± 3.7	0.327	4.1 ± 3.9	3.4 ± 2.6	0.403
neurological problems	8.7 ± 10.5	10 ± 9.5	8.5 ± 8.2	0.235		8.4 ± 8.5	8.9 ± 9.5	0.227	10.2 ± 9.6	7.2 ± 5.7	0.324

EORTC QLQ-C30 – European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (QLQ) Core 30; QLQ-H&N43 – EORTC QLQ Head and Neck Module; S – surgical obturator; I – interim obturator; D – definitive obturator. All results are expressed as mean ± standard deviation. *$p < 0.05$; **$p < 0.01$.

er [18]. Therefore, research on the QoL of patients with maxillectomy would help healthcare professionals to better inform patients about possible implications, risks, and benefits after maxillectomy.

Even though a large number of studies have investigated QoL after treatment for malignant cancer in the face region, the number of studies dealing with the QoL of patients after maxillectomy and OP rehabilitation is limited [9]. The EORTC QLQ-C30 and QLQ-H&N43 are well-established questionnaires for measuring the HNC patient's perception of problems and well-being [15].

It is reported that the female patients showed significantly lower global health status after maxillectomy and rehabilitation with OPs compared to male patients [12]. Similarly, in our subjects, from all functioning scales, the lowest score, in both sexes, was the global health status, which was more pronounced in females.

HNC has been described as one of the most emotionally traumatic types of cancer that can lead to increased levels of anxiety and depression [19, 20]. In our report, female respondents had significantly worse scores in emotional functioning, which is in accordance with the other analyzed reports [14]. Similar to the findings of previous studies, it appeared that women had more pronounced symptoms such as fatigue, insomnia, and appetite loss in the study presented [8, 9, 21].

Male respondents had the lowest score in social functioning, which indicates that the disease and treatment have significantly affected family relationships and relationships with other people. Furthermore, our results have shown a higher rate of financial and sexual problems in male participants, a finding similar to those of previous studies in patients with oral cancer [20, 22].

The study has shown that older cancer patients experience less distress related to cancer treatment as compared to younger patients and that there is an inverse relationship between age and psychological distress after rehabilitation [8, 23]. In the current study, younger patients had worse QoL in many aspects compared to older patients, a finding similar to those of previous studies [9, 23]. This can be explained by the fact that older patients better accept age-related physical illness due to the natural course of life and comorbidity associated with advancing age. Likewise, younger patients are not well prepared for serious illnesses and may feel that their life span has been shortened and their QoL disturbed because of the disease [23, 24]. Some other authors have come to the opposite results and reported that younger patients had better QoL after maxillectomy and rehabilitation with OP [11, 12].

Previous investigations have acknowledged that the presence of caring family members, socioeconomic advantages, and community life may contribute to a better perception of QoL [8]. In this study, the singles were more compromised in physical functioning, fatigue, and financial difficulties. The study has shown that a companionless lifestyle, which is associated with social isolation and increased depression and anxiety, can cause worse physical or emotional fatigue [12]. In addition to the burden of the basic disease, the lack of assistance in daily obligations

significantly physically burdens patients who live alone. Moreover, the socioeconomic aspect of life in partnership makes it easier for patients to overcome the economic problems caused by the disease, especially in developing countries.

Studies reported that HNC patients have enormous problems before dental rehabilitation [3, 5]. Some authors reported certain improvements in oral functions: chewing, swallowing, mouth opening, speech, orofacial appearance, and social interaction after prosthetic rehabilitation with definitive OP [7, 8, 25, 26]. Assessments with the EORTC QLQ-C30 questionnaire showed discreet improvements in all functional scales during rehabilitation. The QoL parameters vary depending on the type of OP. The global health status in the EORTC QLQ-C30 was the best rated in patients with definitive obturators compared to patients with surgical and interim obturators, which is in accordance with a recent study [27].

The following difficulties have been reported as the most common problems that occur after the invasive maxillectomy procedure: difficulties in social and physical contact with others, public appearance, communications with people either directly or by telephone, as well as dining in front of family members and other people [23]. The surgical obturator does not have the best obturation characteristics, which leads to nasal regurgitation and problems with speech and eating [27]. That was the reason for the high expression of dry mouth, problems with speech, social eating, and loss of appetite in patients with surgical obturators in relation to the condition after definitive rehabilitation. Fatigue and insomnia were also dominant side effects in patients with surgical obturators compared to other expected side effects. That has already been confirmed in other patients with HNC immediately after surgical treatment [28]. Another dominant symptom in the early phase of rehabilitation is constipation which can be attributed to previous interventions and difficulties in food intake [29].

Prosthetic rehabilitation of surgically treated cancer of the maxilla and maxillary sinus is completed by making a definitive OP. In our study, the wide range of time periods for definitive prosthetic treatment may be due to the different duration of pronounced side effects of radiation and the time required for the improvement of the general condition. Several studies reported that function and symptoms after definitive rehabilitation return to the level from before the surgical intervention [8, 27]. Our results indicate significant improvement in functioning and symptoms after definitive prosthetic rehabilitation, especially in patients who have some of their own teeth, compared to patients with total obturator dentures or in stage with surgical or interim OP. That is in agreement with other reports because lack of teeth makes speech and mastication difficult [8, 23, 27].

The more pronounced symptoms have been observed among irradiated patients. Problems with the senses, dry mouth, and skin are a direct consequence of radiotherapy, as confirmed in other studies [10, 30].

There are several limitations in this study that should be acknowledged. First, the sample size of this study was relatively small and was carried out in a single medical center, which is why the results should be viewed as preliminary; therefore, further research is needed.

In addition, the limitation of this study is the fact that it does not represent temporal changes in QoL from the moment of obtaining the surgical obturator to final reconstruction with definitive obturators; instead, the study shows QoL in different patients. The additional disadvantages were the lack of data on preoperative QoL assessment, size, and classification of the postoperative defect and occlusion characteristics before prosthetic rehabilitation. Finally, participants restored with implant-retained OPs were excluded from this study.

So far, the investigation of QoL in the Serbian population with malignant tumors in the head and neck region has been already implemented [15, 20, 31]. However, this study is, to the best of our knowledge, the first to investigate the health-related QoL in this population after prosthetic rehabilitation.

Conclusion

The results of this study suggest that sex, age, marital status, and irradiation therapy had a significant impact on QoL. Furthermore, the definitive prosthetics rehabilitation with partial denture significantly improves QoL in patients after maxillectomy. Using this QoL questionnaire in clinical practice would help healthcare professionals understand the impact that the disease and its treatment have on patients' lives. Still, there is a further need for a prospective longitudinal trial with a larger sample to identify predictors of QoL in patients with maxillary defects after rehabilitation.

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Myeloid-derived suppressor-like cells – a potential biomarker for prognosis of colorectal cancer?

Ćelije nalik supresorskim ćelijama mijeloidnog porekla – potencijalni biomarker za prognozu kolorektalnog karcinoma?

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Abstract

Background/Aim. Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous group of immature cells that have the ability to inhibit both the innate and adaptive immune response. Due to their immunosuppressive effect, MDSCs can promote the growth and progression of cancer. Colorectal cancer (CRC) is one of the most common cancers in the general population for whose advanced stages there is still no successful therapy. In addition to contributing to the development and spread of CRC, MDSCs could potentially be seen as markers of its prognosis. The aim of the study was to examine the potential prognostic role of peripheral blood MDSC counts in CRC patients. **Methods.** This prospective study analyzed the possibility of using $CD16^{low}$ granulocytes and monocytic MDSC (M-MDSC) like cells, as well as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), $CD16^{high}/CD16^{low}$ granulocytes ratio, and monocyte-to-M-MDSC like cells ratio, before the start of the treatment, as biomarkers for overall survival (OS) in patients with CRC. The hazard ratio with the corresponding confidence interval of 95% (95% CI) was calculated to evaluate the prognostic role of MDSC in CRC. **Results.** The analysis was performed in 47 patients with stages III and IV of CRC according to the TNM/AJCC disease classification. Reliable data were obtained from 32 patients. Patient blood samples were taken before the possible start of treatment (surgery, chemotherapy). Increased percentages and absolute values of $CD16^{low}$ granulocytes, as well as absolute values of M-MDSC like cells, were associated with shorter OS ($p < 0.0066$, $p < 0.0013$, and $p < 0.0119$, respectively). The relationship between $CD16^{high}/CD16^{low}$ granulocytes ratio and monocyte/M-MDSC like cells ratio with OS indicated the existence of positive correlations between these parameters, where the higher value of this relationship indicated longer OS of patients ($p < 0.0054$ and $p < 0.0148$, respectively). The relationship between OS and NLR showed a statistically significant inverse correlation ($p = 0.0349$). No statistical significance was found in the relationship between OS and LMR. **Conclusion.** Percentages and absolute numbers of $CD16^{low}$ granulocytes, as well as the absolute numbers of M-MDSC like cells, the $CD16^{high}/CD16^{low}$ granulocytes ratio, monocyte/M-MDSC like cells ratio, and NLR ratio, may be reliable indicators of OS in patients with CRC.

Keywords:
biomarkers; colorectal neoplasms; myeloid-derived suppressor cells; prognosis; treatment outcome.

Apstrakt

Uvod/Cilj. Supresorske ćelije mijeloidnog porekla (SĆMP) predstavljaju heterogenu grupu nezrelih ćelija, koje imaju sposobnost da inhibiraju i urođeni i stečeni imunski odgovor. Zbog svog imunosupresivnog efekta one mogu da podstiču rast i progresiju karcinoma. Kolorektalni karcinom (KRK) je jedan od najčešćih karcinoma u opštoj populaciji za čije odmakle stadijume još uvek ne postoji uspešna terapija. Osim što doprinose razvoju i širenju KRK, SĆMP bi mogle potencijalno biti i markeri njegove prognoze. Cilj rada bio je da se ispita potencijalna prognostička uloga broja SĆMP periferne krvi u KRK. **Metode.** U prospektivnoj studiji analizirana je mogućnost upotrebe $CD16^{slabo+}$ granulocita i ćelija nalik monocitnim SĆMP (M-SĆMP), kao i odnosa neutrofila i limfocita (*neutrophyl-to-lymphocyte ratio* – NLR), limfocita i monocita (*lymphocyte-to-monocyte ratio* – LMR), odnosa $CD16^{jako+}/CD16^{slabo+}$ granulocita i odnosa monociti/ćelije nalik M-SĆMP, merenih pre početka tretmana, kao biomarkera za ukupno preživljavanje (UP) kod bolesnika sa KRK. U proceni prognostičke uloge SĆMP u KRK

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korišćen je parametar odnos rizika, uz odgovarajući interval poverenja od 95%. **Rezultati.** Analizirano je 47 bolesnika u III i IV stadijumu KRK, prema TNM/AJCC sistemu klasifikacije bolesti. Pouzdani podaci dobijeni su od 32 bolesnika. Uzorci krvi bolesnika bili su uzeti pre eventualnog započinjanja lečenja (operacija, hemioterapija). Pokazano je da su povišene relativne i apsolutne vrednosti $CD16^{slabo+}$ granulocita kao i apsolutne vrednosti ćelija nalik M-SĆMP bile povezane sa kraćim UP ($p < 0,0066$, $p < 0,0013$ i $p < 0,0119$, redom). Veza između odnosa $CD16^{jako+}/CD16^{slabo+}$ granulocita kao i odnosa monociti/ćelije nalik M-SĆMP i UP, ukazala je na postojanje pozitivne korelacije između tih parametara, pri čemu je viša vrednost korelacije ukazivala na duže UP bolesnika ($p < 0,0054$ i $p < 0,0148$, redom). Između UP i NLR nađena je statistički značajna inverzna korelacija ($p = 0,0349$). Nije potvrđena statistički značajna povezanost između UP i LMR. **Zaključak.** Relativne i apsolutne vrednosti $CD16^{slabo+}$ granulocita, kao i apsolutne vrednosti ćelija nalik M-SĆMP, odnos $CD16^{jako+}/CD16^{slabo+}$ granulocita, odnos monociti/ćelije nalik M-SĆMP i NLR, mogu biti pouzdani pokazatelji UP kod bolesnika sa KRK.

Ključne reči:
biomarkeri; kolorektalne neoplazme; kostna srž, ćelije, supresorske; prognoza; lečenje, ishod.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer (after breast and lung cancer) and the second leading cause of death due to malignant disease [1]. In Europe, Hungary leads in the incidence of CRC with 51.2 cases *per* 100,000 inhabitants [2]. CRC is more common in men than women. It can develop as part of hereditary diseases such as familial adenomatous polyposis, Lynch syndrome, and Peutz-Jeghers syndrome but also as a consequence of inflammatory bowel diseases (e.g., ulcerative colitis). It most often occurs in the population as a sporadic disease, the occurrence of which is affected by older age, obesity, alcohol and cigarette consumption, excessive use of red meat and meat products, long-term use of androgens, diabetes, and even cholecystectomy [3, 4].

To better understand the nature of cancer, it is necessary to understand the complex relationships that exist within the tumor microenvironment (TME). TME is an environment composed of cells that build extracellular space and blood vessels, but also of immunologically active cells that belong to the innate and adaptive immune response, fibroblasts associated with cancer, as well as a large number of signaling molecules that are important in cancer formation, maintenance, and dissemination [5]. An important place in TME is also occupied by myeloid-derived suppressor cells (MDSCs), immature cells of myeloid origin with an immunosuppressive effect. MDSCs were first described in the 1970s in mice in which they were first phenotypically defined [6]. They were labeled as $Gr1^+CD11b^+$ cells. Two subgroups of these cells were then observed, polymorphonuclear (PMN) MDSCs (PMN-MDSCs), with surface markers CD11b and Ly6G, and monocytic (M) MDSCs (M-MDSCs), with markers CD11b and Ly6C [7]. At the same time, efforts have been made to define these cells phenotypically in humans. Due to the heterogeneity of these cells, this process is still a challenge today, and, in addition to phenotypic identification, molecular and functional determination of these cells is increasingly used. Today, these cells in humans are usually defined as $CD14^-CD15^+CD11b^+CD33^+HLA\text{-}DR^-lin^-$ and $CD11b^+CD14^-CD66b^+$, which is a phenotype shared by mature neutrophils as well and represent PMN-MDSCs and $CD14^+CD15^+CD11b^+CD33^+HLA\text{-}DR^-Lin^-$ which are similar to monocytes and represent M-MDSCs [8]. Lectin-type oxidized LDL receptor 1 (LOX 1) is increasingly mentioned in the literature as a marker of PMN-MDSCs in humans that allows better differentiation between neutrophils and PMN-MDSCs without the use of a gradient separation [9]. In recent years, another type of MDSCs has been mentioned in the human population, which is present in a small percentage. That type is called early-stage MDSCs (e-MDSCs), which lack markers for both monocyte and granulocyte populations, whose phenotype is $Lin^-HLA\text{-}DR^-CD33^+CD11b^+CD14^-CD15^-$, and contain immature progenitor and precursor cells [8, 10]. It should be emphasized that MDSCs are not cells that are exclusively located in the TME but are also present in the peripheral circulation in patients with malignant diseases. These cells can also be found in the peripheral blood (PB) of apparently healthy people as well as in many other physiological and pathological conditions unrelated to malignancy. The development and functional roles of MDSCs in various conditions, including pregnancy, inflammatory diseases of various etiologies, trauma, autoimmune diseases, heart failure and acute coronary syndrome, obesity, sepsis, Alzheimer's disease, Parkinson's disease, etc., are discussed in detail in other studies [11–20].

By now, it is well known that the circulating and tumor-infiltrating MDSCs play an important role in CRC [21, 22]. It is also accepted that the MDSCs accumulate in the late stage of malignant diseases and correlate positively with the disease progression [11]. Nevertheless, recent findings of Ma et al. [10] suggest that MDSCs accumulate even in premalignant lesions, such as colon polyposis and intraductal papillary mucinous neoplasm, and that these cells share phenotypic and functional characteristics with MDSCs seen in overt neoplasms. The development and maintenance of MDSCs in malignant diseases, including CRC, is influenced by numerous mediators released under different chronic inflammatory processes [11, 23]. These mediators encompass different chemokines and growth factors, inflammatory mediators (histamine, prostaglandins, and leukotrienes), as well as local hypoxia and low pH present within the TME [11, 24–27]. Regarding the MDSCs development in CRC, one of the particularly important factors is CCL2 which has been shown to cause MDSCs accumulation and enhance their immunosuppressive function during colorectal carcinogenesis [28]. The mechanism

of MDSCs action in CRC is also well described and relies mainly on their ability to inhibit T cell function through reactive oxygen species production and inducible nitric oxide synthase activity and to stimulate regulatory T cell development [11, 29]. These cells also promote CRC growth by releasing the exosomes containing the S100A9 protein within TME, which was recently shown for PMN-MDSCs by Wang et al. [30].

There is a considerable amount of data in the literature that connect either: the MDSCs with the clinical stage and spread of CRC disease [31]; the frequency of MDSCs in the paraffin-embedded sections with survival prediction [32]; pretreatment levels of MDSCs in the PB with progression-free survival (PFS) in patients with unresectable metastatic CRC [33] or with overall survival (OS) in patients with resectable CRC prior to surgical therapy, but without separate analysis of the two main subsets of the MDSCs [34]. In addition, the neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) are widely used as prognostic indicators in CRC [35, 36]; however, we could not find any data regarding the prognostic value of ratios within the related cell populations such as neutrophils and PMN-MDSCs as well as monocytes and M-MDSCs in CRC patients.

The aim of our study was to examine the potential prognostic role of PB MDSC counts in CRC patients considering both main subtypes of these cells (PMN-MDSCs and M-MDSCs), as well as to analyze the prognostic usefulness of ratios within the related cell populations such as neutrophils and presumable PMN-MDSCs as well as monocytes and presumable M-MDSCs, in parallel with widely used NLR and LMR indicators in CRC patients. Given that we used fresh and lysed PB samples, in which the MDSCs gating strategy is still insufficiently defined, we designated our cells of interest as MDSC-like cells. The following text's abbreviations $CD16^{low}$ granulocytes and M-MDSC like cells refer to phenotypically matched, presumable PMN-MDSCs and M-MDSC, respectively.

Methods

Patients

The study included 47 patients diagnosed with stage III (18 patients) and stage IV (29 patients) of CRC, according to the last, eighth TNM/AJCC classification. The study was approved by the Ethics Committee of the Military Medical Academy (MMA), Belgrade (from March 10, 2016), and every patient provided a signed consent form. After the diagnosis of CRC, a blood sample was taken from the patients at the Clinic for Gastroenterology and Hepatology, MMA, Belgrade, Serbia. The patients were then monitored from June 2016 until May 2020. Patients whose eventual lethal outcome was the consequence of some other diseases or who did not report for regular follow-up examinations were excluded from the study. Sample processing was performed at the Institute for Medical Research, MMA, Belgrade. Results were obtained for 32 patients.

Samples

Three mL of venous blood were sampled from the patients with CRC. Erythrocytes were removed by lysis (EDTA, NH_4Cl, $KHCO_3$) for 20 min with constant stirring. A double wash of the nucleated cells in culture medium (RPMI640) with 5% normal human serum was performed with subsequent centrifugation and resuspension. Separation of PB mononuclear cells for comparative analysis was performed using LSM 1077 lymphocyte separation medium. Separation was performed by centrifugation at 1,200 × g for 20 min. The interphase layer was separated and washed twice in the culture medium. The number of cells was determined automatically on the Beckman Coulter ACT diff blood cell counter. The cells were resuspended at a final concentration of 1×10^6 *per* 100 μL of the suspension for further staining. After an initial comparison of the yield of the cells with the below-described phenotype between lysed samples and samples obtained with gradient centrifugation, we decided to continue the analysis with lysed samples only in order to avoid cell loss and determine the frequency and an absolute number of $CD16^{low}$ granulocytes and M-MDSC-like cells in all study participants.

Immunophenotyping of cells

The following human monoclonal antibodies were used to perform cell immunophenotyping: CD15-FITC and PECy7, CD33-PE and PECy7, CD45-ECD, HLA-DR PECy5, CD14-PECy7, CD16-FITC and PECy7, CD11b-PE, CD10-PECy7, CD3-FITC; CD19-FITC and CD56-FITC (Beckman Coulter, USA). Stained cells were analyzed on a Beckman Coulter FC 500 flow cytofluorimeter using CXP analytical software. MDSC-like cells were defined as $Lin^-(CD3^-/CD19^-/CD56^-)/HLA\text{-}DR^{-/low}CD11b^+$ cells. Polymorphonuclear and monocyte subtypes were determined based on the expression of CD14 and CD15. $CD16^{low}$ granulocytes were defined as $CD14^-CD15^+$ and M-MDSC-like cells as $CD14^+CD15^-$. The gating strategy for detection and enumeration of MDSC-like cells was based on previous work by Stanojević et al. [37]. It should be noted here that the $CD16^{low}$ granulocyte gating strategy based on CD16 expression may be a pitfall because changes in CD16 expression may also be due to changes in the functional status of healthy, mature neutrophils [38]. However, lower expression of CD16 can still be an indicator of granulocyte immaturity and/or pathological activation, and these characteristics in PB granulocytes could be attributed to PMN-MDSCs [39]. Indeed, in capecitabine-resistant CRC patients, Lu et al. [40] showed that $CD16^{low/-}$ neutrophils exerted immature gene expression profile and metabolic activity tightly related to the immunosuppressive role of MDSCs, as well as their direct immunosuppressive effects in T cell proliferation test.

Statistical analysis

All statistical analyses were performed in GraphPad Prism 9.0.2. In this study, we conducted a correlation analy-

sis to measure the level of correlation between the two variables. If the data distribution corresponded to the normal distribution, Pearson's coefficient was used to examine the degree of the linear relationship between two (numerical) variables. On the other hand, Spearman's correlation coefficient was used if the data did not have a normal distribution, was measured on an ordinal scale, or if the relationship between measured values was not linear.

Results

Immunophenotype and subsets of the MDSC-like cells in CRC patients and healthy controls

$CD16^{low}$ granulocytes and M-MDSC-like cells were identified according to the expression of CD15 and CD14, respectively, within the $HLA\text{-}DR^{-/low}CD11b^{+}CD33^{low}Lin^{-}$ population in all 47 patients in stage III and IV of the disease according to the AJCC classification. The detected immunophenotype of the targeted MDSC-like populations is given in Table 1 [37].

In brief, for detecting $CD16^{low}$ granulocytes, within the granulocyte region on side scatter (SS), cells with low and non-homogeneous expression of CD16 were gated and colored in black for tracking on other dot plots (Figure 1A) and subsequently analyzed for expression of CD11b and HLA-DR (Figure 1B), as well as for expression of CD15 (Figure 1C). On the CD45 vs. SS dot plot, black-colored cells with low and nonhomogeneous expression of CD16 showed lower levels of CD45 expression (Figure 1D) vs. eosinophils which are clearly a CD16 negative (black colored, Figure 2A) homogeneous population with strong expression of CD45 molecule (Figure 2B).

For detecting M-MDSC-like cells, the cells with positive CD11b and negative/low HLA-DR expression (Figure 3A) were checked for CD14 expression and colored black on CD14 vs. SS log dot plot for further tracking (Figure 3B). In the next step, the expression of lineage markers CD33 and CD15 was examined. The cells with $HLA\text{-}DR^{-/low}CD11b^{+}CD14^{+}CD33^{+}CD15^{-}Lin^{-}$ immunophenotype were designated and enumerated as M-MDSC-like cells. As the internal control, we used lymphocytes to compare HLA-DR (Figure 3C) and CD14 expression (Figure 3D).

Table 1

Immunophenotype of detected myeloid-derived suppressor cell (MDSC) like populations

MDSC-like subset	HLA-DR	CD3	CD10	CD11b	CD14	CD15	CD16	CD19	CD33	CD45	CD56
$CD16^{low}$ granulocytes	-/low	-	-	+	-	+	low/int	-	low	low	-
M-MDSC-like cells	-/low	-	-	+	+	-	-/low	-	low	low	-

$CD16^{low}$ granulocytes and M-MDSC-like cells were identified according to the expression of CD15 and CD14 within the $HLA\text{-}DR^{-/low}CD11b^{+}CD33^{low}Lin^{-}$ population.
M-MDSC – monocytic MDSC.
Modified Table 1 [37].

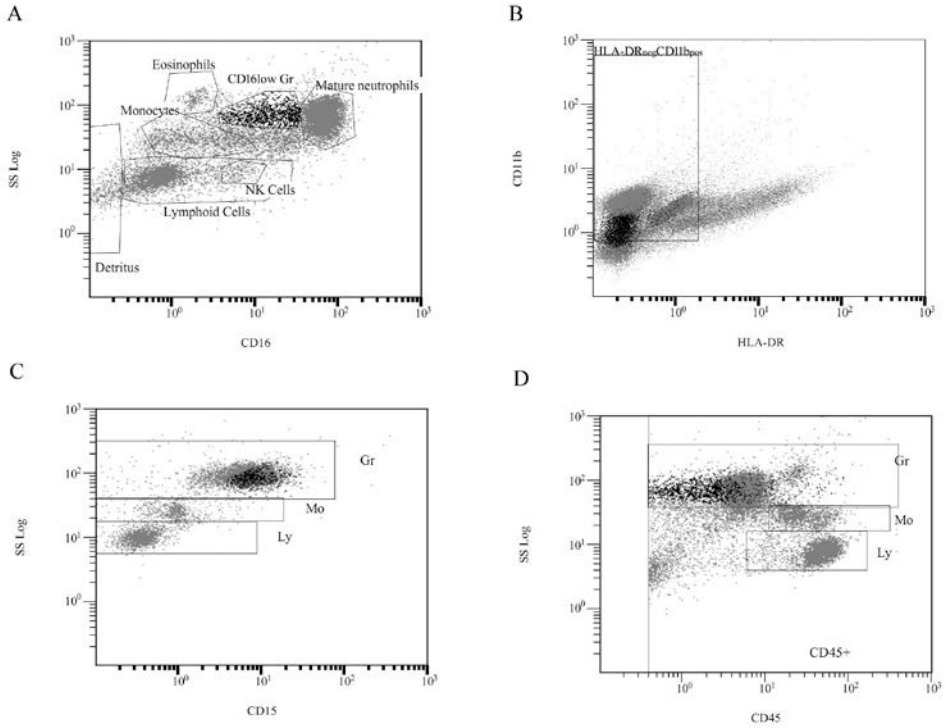

Fig. 1 – Detection of $CD16^{low}$ granulocytes (Gr) within the granulocyte region on side scatter (SS) in relation to A) CD16 expression, B) HLA-DR and CD11b expression, C) CD15 expression, and D) CD45 expression.
NK – natural killer; Mo – monocytes; Ly – lymphocytes.

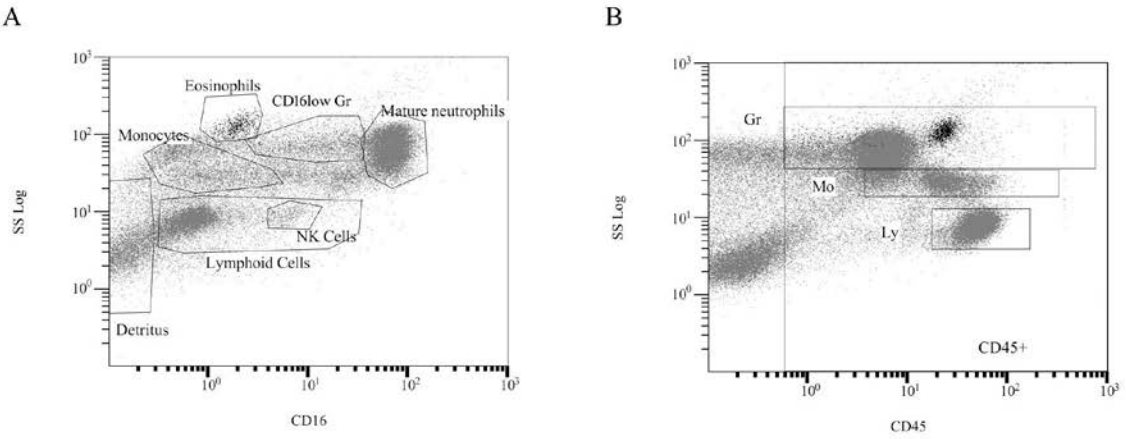

Fig. 2 –Detection of CD16^{low} granulocytes (Gr) in relation to: A) eosinophils, monocytes, natural killer (NK) cells, lymphoid cells, and mature neutrophils and to B) monocytes (Mo), lymphocytes (Ly), and granulocytes (Gr).

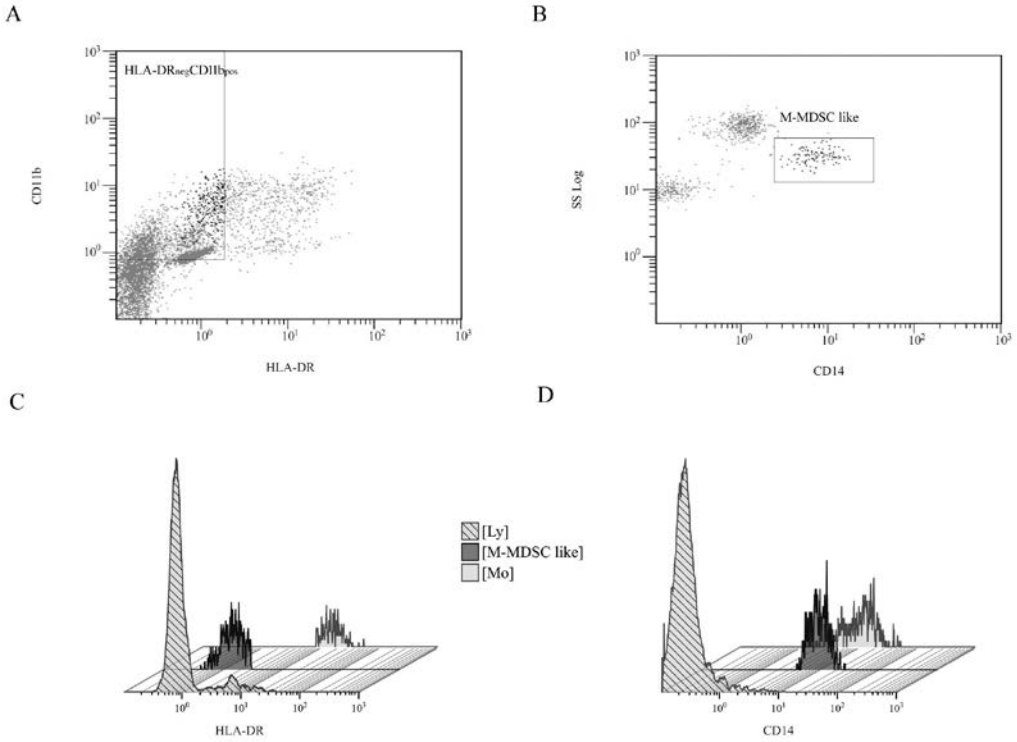

Fig. 3 – Detection of monocytic myeloid-derived suppressor cell (M-MDSC)-like cells on side scatter (SS) in relation to A) HLA-DR and CD11b expression and to B) CD14 expression. Overlay histograms show internal comparison with lymphocytes (Ly) expression of C) HLA-DR and D) CD14. Mo – monocytes.

Correlation between CD16^{low} granulocytes, M-MDSC-like cells, and overall survival

Relevant OS-related data were obtained from 32 patients. The average age of the patients was 71.9 years (men – 66.4 years, women – 77.3 years). Patients were followed for three years from the diagnosis of CRC and from taking the first sample. Spearman's correlation coefficient indicated the presence of a moderately negative linear relationship between OS measured in days and M-MDSC-like cells prevalence (Table 2). It follows from the above that patients with shorter OS had a higher percentage and an absolute number of CD16^{low} granulocytes (Spearman r = -0.4705, $p < 0.0066$ and Spearman r = -0.545, $p < 0.0013$, respectively) (Table 2) (Figure 4 A–B). For the M-MDSC-like cells, statistical significance was observed only for the absolute number of these cells (Spearman r = -0.4394, $p < 0.0119$) (Table 2) (Figure 4 C–D).

Table 2

Spearman's correlation of CD16^{low} granulocytes and M-MDSC-like cells with overall survival

	OS and CD16^{low} granulocytes		OS and M-MDSC-like cells	
	%	No/μL	%	No/μL
Spearman coefficient	-0.4705	-0.545	-0.3081	-0.4394
p-value	0.0066	0.0013	0.0863	0.0119
Significant (α = 0.05)	yes	yes	no	yes

M-MDSC – monocytic myeloid-derived suppressor cell.

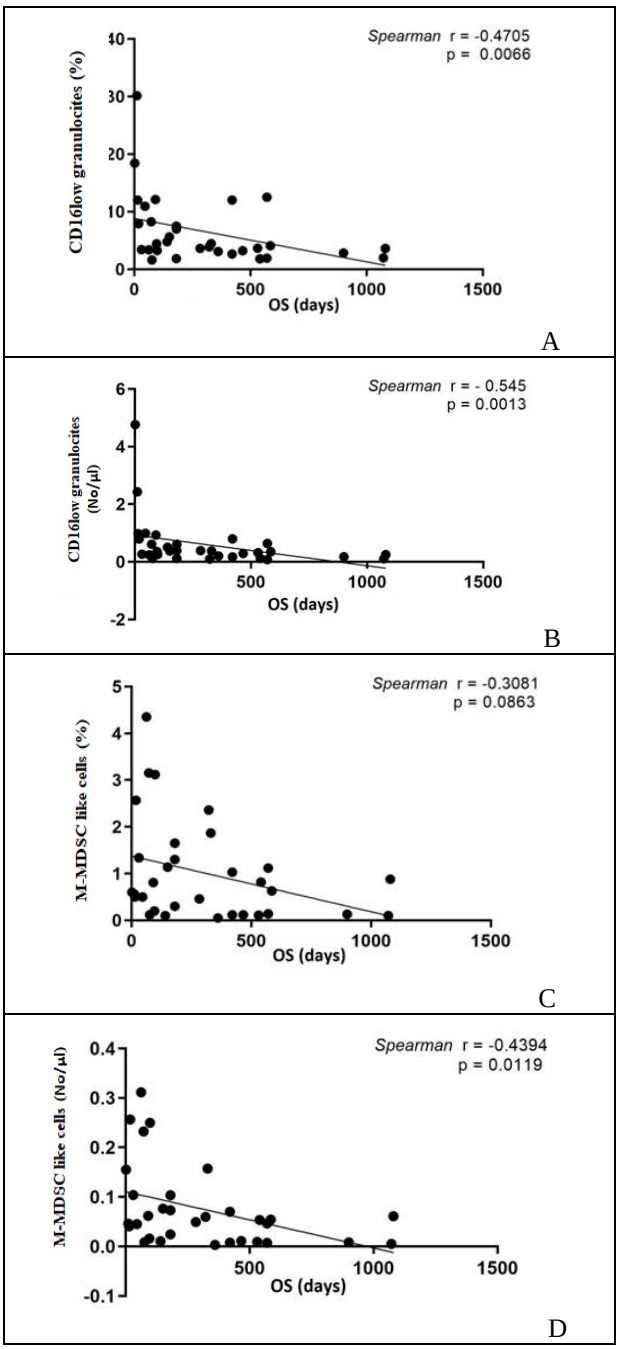

Fig. 4 – Percentages (A) and absolute numbers (B) of CD16^{low} granulocytes in relation to overall survival (OS). Percentages (C) and absolute numbers (D) of M-MDSC-like cells in relation to OS.
M-MDSC – monocytic myeloid-derived suppressor cell.

Correlation between NLR, LMR, CD16^{high}/CD16^{low} granulocytes ratio and monocyte/M-MDSC-like cells ratio and overall survival

In the same follow-up time of three years, we analyzed the relationship between OS and NLR and between OS and LMR and found a statistically significant inverse correlation with NLR (Spearman r = -0.3741, p = 0.0349) (Figure 5A). No statistical significance was found in the relationship between OS and LMR (Spearman r = 0.3328, p = 0.0627) (Figure 5B). We also tried to determine the possible connection of CD16^{high}/CD16^{low} granulocytes ratio and monocyte/M-MDSC-like cells ratio with OS. Spearman's correlation coefficient was tested first, then its significance for both ratios was calculated (Table 3). The study showed a moderately positive correlation between OS and CD16^{high}/CD16^{low} granulocytes ratio (Spearman r = 0.4802, p = 0.0054) (Figure 5C) as well as between OS and monocyte/M-MDSC-like cells ratio (Spearman r = 0.4269, p = 0.0148) (Figure 5D).

Discussion

One of the most important and common categorizations used in determining the stage and prognosis of CRC disease is the TNM/AJCC classification. However, this classification requires knowledge of the degree of invasion of the intestinal wall and lymph glands as well as the possible presence of metastases. Even though the TNM classification is the most important for assessment in clinical practice, many researchers have tried to predict the outcome of the disease or the potential response to therapy with a simpler approach and quick orientation. The NLR was initially used to assess

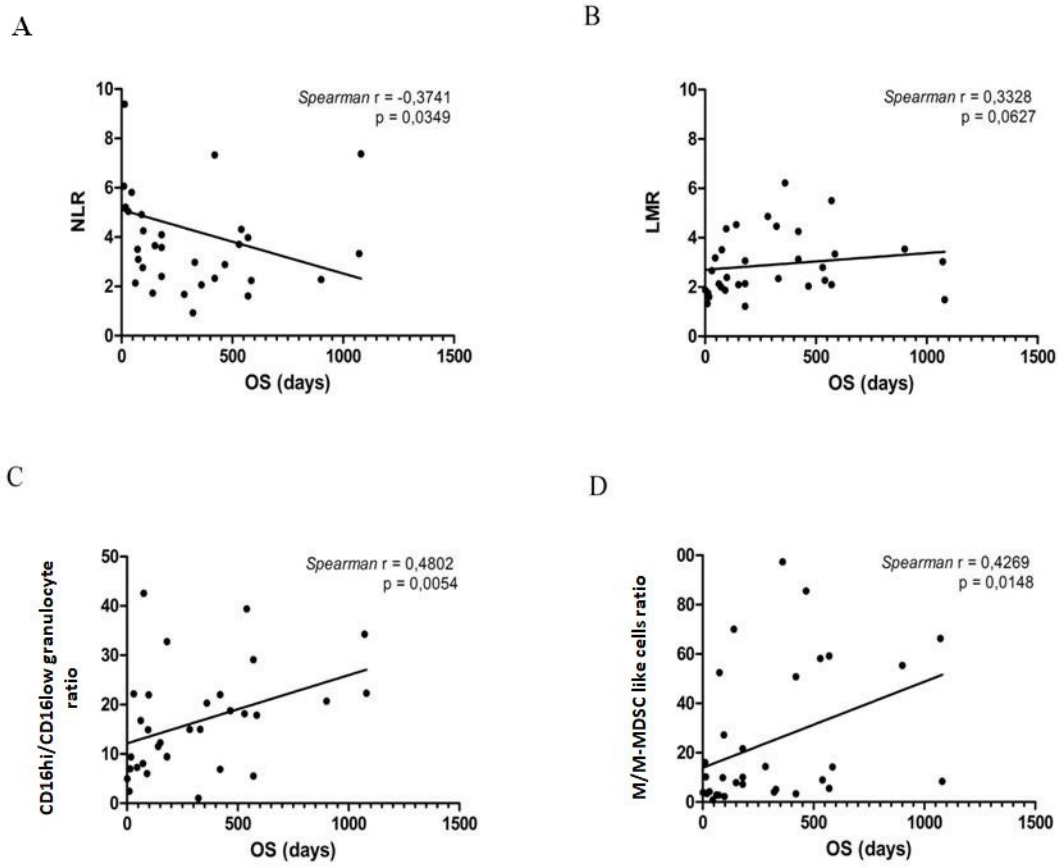

Fig. 5 – Correlation between overall survival (OS) and A) NLR, B) LMR, C) CD16^{high}/CD16^{low} granulocytes ratio, and D) M/M-MDSC-like cells ratio. M/M-MDSC – monocyte/monocytic myeloid-derived suppressor cell; NLR – neutrophil-to-lymphocyte ratio; LMR – lymphocyte-to-monocyte ratio. One data point in A) NLR is out of the y-axis range (y = 21.17, x = 1).

Table 3

Spearman's correlation between overall survival (OS) and CD16^{high}/CD16^{low} granulocytes ratio and the M/M-MDSC-like cells ratio

	OS and CD16^{high}/CD16^{low} granulocytes ratio	OS and M/M-MDSC-like cells ratio
Spearman's coefficient	0.4802	0.4269
p-value	0.0054	0.0148
Significant (α = 0.05)	yes	yes

M/M-MDSC – monocyte/monocytic myeloid-derived suppressor cell.

outcomes in patients with inflammation in the intensive care unit. Today, it often serves as a predictor of prognosis in many malignancies [35]. NLR is of special importance because it can be easily determined only from a patient's blood count sample. This method also has its limitations because the increase in this ratio does not only indicate a poor outcome for a patient who has a malignant disease but can also occur as a consequence of other diseases of the patient [41]. In recent years, both LMR and platelet-lymphocyte ratio have been used [36].

So far, in addition to these known prognostic relationships, several studies have examined the prognostic role of MDSCs in CRC patients [32–34]. The presence of circulating MDSCs in various types of solid organ cancers has been most commonly examined. Circulating MDSCs were chosen rather than the tumor-infiltrating MDSCs because they are technically easier to determine. Most patients in stage IV of the disease do not undergo surgical removal of the cancer and R0 resection but only a biopsy of the observed tumor. Likewise, further sampling and comparison of MDSC values after the tumor tissue is removed are possible only from the circulation. However, it is necessary to emphasize here that the determination of MDSCs in PB is only an indirect parameter that does not have to faithfully reflect the situation in the TME.

The association between MDSCs and the stage of disease in CRC is known [29, 42]. We tried to assess the MDSC frequencies and their absolute numbers as a potential prognostic biomarker in patients with this malignancy. A negative linear relationship between survival and MDSCs prevalence has been observed in several studies and is thought to be a consequence of their immunosuppressive function [43]. Our results confirmed that patients with shorter OS correlated positively with a higher percentage and absolute number of $CD16^{low}$ granulocytes before surgery. By M-MDSCs testing, statistical significance was observed only in the absolute number of M-MDSC-like cells. A study by Lang et al. [44], which included patients with head and neck cancer, found that patients with a high percentage of PMN-MDSCs had a significant reduction in survival and the strongest immunosuppression of T cells, while high M-MDSC values indicated a worse prognosis but not at the level of statistical significance.

Following the previously observed negative correlation demonstrated in our study, we attempted to determine whether there is an association between $CD16^{high}/CD16^{low}$ granulocytes ratio as well as monocyte/M-MDSC-like cells ratio with the OS of our patients in stage III and IV of CRC according to the TNM classification. As mentioned above, many studies dealt with NLR or LMR associations with OS in different cancer types, including CRC; however, according to the same database, we found only one study that analyzed relationships within related MDSC populations. In that study, Sheng et al. [45] found that the uncommitted MDSC/PMN-MDSCs ratio showed an inverse correlation with NLR and poorer OS outcomes in urothelial carcinoma patients with high levels of uncommitted MDSCs. In our study, we attempted to analyze relationships within related cell populations such as $CD16^{high}/CD16^{low}$ granulocytes ratio as well as monocytes and M-MDSC-like cells. In this regard, we observed that the $CD16^{high}/CD16^{low}$ granulocytes ratio and monocyte/M-MDSC-like cells ratios showed a positive correlation with OS indicating the connection between lower MDSC prevalence and better disease outcome. The reason why we decided to analyze the internal relationships within phenotypically determined related populations is that the mentioned NLR or LMR are calculated based on the results of automated blood counters that cannot recognize subtle differences between individual cells within a related population. For instance, it is generally accepted that high NLR and/or low LMR in tumor patients, including CRC patients, are associated with poor outcomes [46]. However, it is also known that the composition of circulating neutrophils in tumor patients is diverse and includes high-density neutrophils, which are thought to have anti-tumor properties and correspond to N1 neutrophils in TME, as well as low-density neutrophils with pro-tumor effects, which correspond to N2 [47]. Similarly, at least three subsets can be phenotypically identified among circulating monocytes: classical ($CD14^{high}CD16^{-}$), nonclassical ($CD14^{+}CD16^{+}$), and intermediate subset ($CD14^{low}CD16^{+}$), which may have opposite roles in tumor development, growth, and metastasis, including CRC [48]. Indeed, in our study, we found that the $CD16^{high}/CD16^{low}$ granulocytes ratio showed an even better Spearman's rank coefficient compared to NLR, while in LMR correlation analysis, the statistical significance was not achieved at all, unlike the monocyte/M-MDSC-like cells ratio which showed a statistically significant positive correlation with OS. It is known that the number of neutrophils can increase in patients with malignant disease as a consequence of chronic inflammation, as well as the relative and absolute values of MDSCs [49]. Similarly, many studies have shown that the prevalence of MDSCs increases with the stage of the disease, and the more severe stage of the disease indicates a worse prognosis and shorter survival [50–52]. The number of PMN-MDSCs and M-MDSCs in the advanced stages of the disease still increases more than the number of neutrophils and monocytes. There are at least three mechanisms responsible for the increases in the relative and absolute values of both MDSC subtypes, which include the urgent myelopoiesis in response to the existence of cancer, the plasticity of myeloid cells, and extramedullary myelopoiesis and are explained in more detail in other studies [50, 53, 54]. Regardless of the dominant mechanism, as a consequence, there is an increase in the frequency of both subtypes of MDSC, which is more pronounced in the advanced stages of the disease.

However, correlations between the relationship of $CD16^{high}/CD16^{low}$ granulocytes ratio and monocyte/M-MDSC-like cells with OS, as well as the simple prevalence of MDSCs in PB, should be viewed in a broader light of an individual patient's immune status. In their study on CRC patients, Tada et al. [33] showed that a high proportion of M-MDSCs in PB was associated with significantly shorter PFS. However, by examining other subsets of immune cells, namely $CD4^{+}$ and $CD8^{+}$ effector memory T cells (among others), they found that in patients with a high proportion of M-MDSCs, who are expected to have shorter PFS, that was

not the case if high percentages of effector memory T cells were present at the same time, and *vice versa*. The significance of more detailed immune profiling of PB cells in CRC patients was confirmed by other authors as well [55].

We consider that the main disadvantages of our study are the fact that we did not analyze repeated PB samples during the follow-up of the patients and a relatively small number of participants. Parallel analysis of lysed samples and samples obtained by gradient centrifugation should be performed to verify proper gating for $CD16^{low}$ granulocytes.

Conclusion

Our data support the potential use of M-MDSC-like cell detection and enumeration as a prognostic marker for CRC, but further research is needed. Analysis of relationships within related populations, i.e., $CD16^{high}/CD16^{low}$ granulocytes ratio and monocyte/M-MDSC-like cells ratios, have the prognostic potential and could improve the prognostic significance of other established OS indicators in CRC patients.

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The effects of various mouth rinses on enamel bond strength of a universal adhesive system

Uticaj različitih sredstava za ispiranje usta na jačinu veze gleđi sa univerzalnim adhezivnim sistemom

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Abstract

Background/Aim. Although essential oil-containing mouth rinses have some advantages, it is not well-known what effects they have on the bond strength of the universal resin adhesives system to the enamel. The aim of the study was to evaluate the effect of essential oil-containing mouth rinses on the enamel bond strength of the universal adhesive system. **Methods.** A total of 96 bovine incisors were used in the study. The teeth were divided into four different groups according to the control group and three different mouth rinses: Group I (Control) – distilled water, Group II – Listerine Cool Mint (essential oil mouth rinse), Group III – Kloroben (0.12% chlorhexidine gluconate mouth rinse), and Group IV – Oxyfresh (0.05% sodium fluoride mouth rinse). Each group was divided into two subgroups according to the application mode of the universal adhesive (etch-and-rinse mode or self-etch mode) (n = 12). Mouth rinses were applied daily for 30 sec to the enamel surfaces for a month, and the samples were soaked in distilled water. After the shear bond strength (SBS) tests were performed with the universal test machine at a speed of 1 mm/min, the SBS data were statistically analyzed (p = 0.05). **Results.** Two-way ANOVA showed that the enamel bond strength of universal adhesive was not affected by mouth rinse and was significantly affected by the application mode. **Conclusion.** The use of essential oil-containing mouth rinses and other mouth rinses tested in the study is safe in terms of the quality of enamel bonding of the tested adhesive.

Key words:
adhesives; dental enamel; essential oils; mouthwashes.

Apstrakt

Uvod/Cilj. Iako sredstva za ispiranje usta koja sadrže eterična ulja imaju određene prednosti, nije dovoljno poznato kakav efekat ona imaju na jačinu veze gleđi sa univerzalnim adhezivnim sistemom. Cilj rada bio je da se proceni uticaj sredstava za ispiranje usta koja sadrže esencijalno ulje na jačinu veze gleđi sa univerzalnim adhezivnim sistemom. **Metode.** U istraživanju je korišćeno 96 goveđih sekutića. Zubi su podeljeni u četiri različite grupe prema kontrolnoj grupi i tri različita sredstva za ispiranje usta: Grupa I (Kontrolna) – destilovana voda, Grupa II – *Listerine Cool Mint* (sredstvo za ispiranje usta sa esencijalnim uljem), Grupa III – Kloroben (0,12% hlorheksidin glukonat u sredstvu za ispiranje usta) i Grupa IV – *Oxyfresh* (0,05% natrijum-fluorid u sredstvu za ispiranje usta). Prema načinu nanošenja univerzalnog lepka adheziva (protokol nagrizanja i ispiranja ili protokol samonagrizanja), svaka grupa je podeljena u dve podgrupe (n =12). Sredstva za ispiranje usta svakodnevno su se nanosila u trajanju od 30 sekundi na površine gleđi tokom mesec dana, a uzorci su zatim potapani u destilovanu vodu. Nakon ispitivanja „čvrstoće smicanja“, primenom univerzalne ispitne test mašine, pri brzini od 1 mm/min, dobijeni podaci su statistički analizirani (p = 0,05). **Rezultati.** Dvosmernim ANOVA testom pokazano je da ispiranje usta nije uticalo na jačinu veze gleđi sa univerzalnim adhezivom, ali je zato način nanošenja značajno uticao. **Zaključak.** Korišćenje sredstva za ispiranje usta koje sadrži esencijalno ulje, kao i drugih sredstava za ispiranje usta testiranih u ovom istraživanju, pokazalo se bezbedno u pogledu kvaliteta veze gleđi i ispitivanog adheziva.

Ključne reči:
adhezivi; zub, gleđ; ulja, etarska; usta, sredstva za ispiranje.

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Introduction

One of the most common infectious oral diseases in humans is dental caries, whose primary etiology is dental plaque [1]. The basis of the prevention of dental caries is the mechanical removal of dental plaque. However, using antiseptic mouth rinses to make dental plaque less cariogenic is recommended in order to prevent dental caries disease since it is almost impossible to remove dental plaque, and the reformation of plaque is inevitable [2, 3].

Among the many different chemical agents found in mouth rinses, the most recommended ones are those mouth rinses containing fluorides, chlorhexidine, and essential oils [4]. In clinical studies, mouth rinses containing fluoride or chlorhexidine compounds have been found to help control the progression of caries lesions [2, 5]. Furthermore, mouth rinses containing essential oils have been shown to reduce dental plaque formation and gingival inflammation in long- and short-term clinical studies [6–8]. The primary mechanism underlying the clinical effect of this mouth rinse is thought to be microbiocidal. It has been shown in *in vitro* studies that this mouth rinse is capable of killing a wide variety of microorganisms in a short time [9]. It can also reduce bacterial load, slow plaque maturation, and reduce the amount and pathogenicity of plaque [10]. Evidence from clinical studies indicates that chlorhexidine and fluoride mouth rinses, along with essential oils, may have contributed to the prevention of tooth decay disease.

Today, significant improvements in the clinical success of resin adhesive systems play an essential role in the more frequent use of tooth-colored aesthetic direct restorations [11]. Universal adhesive systems constitute the latest resin adhesive class introduced to the market. These adhesives have been developed to overcome the shortcomings of one-step self-etch adhesives [11, 12]. One of the most important advantages offered by universal adhesives is the fact that the same adhesive is suitable for applying both the etch-and-rinse and the self-etch application modes. This versatility of universal adhesive systems allows clinicians to choose the ideal approach based on the condition of the cavity.

Although essential oil-containing mouth rinses have some advantages, it is not well-known what effects they have on the bond strength of the universal resin adhesives system to the enamel. In addition, there is not enough evidence in the literature about the effect of other mouth rinses containing fluoride or chlorhexidine on resin-enamel bonding. Therefore, the aim of the study was to evaluate the effects of mouth rinses containing fluoride or chlorhexidine or essential oil on the enamel bond strength of the universal resin adhesive system with the shear bond strength (SBS) test.

Methods

Study design

The independent variables of the study were as follows: mouth rinse Listerine Cool Mint (essential oil-containing mouth rinse), Johnson & Johnson, New Jersey, USA; Kloroben (0.12% chlorhexidine gluconate-containing mouth rinse), Drogsan Pharmaceuticals, Ankara, Turkey; Oxyfresh (0.05% sodium fluoride-containing mouth rinse), Oxyfresh Inc., Idaho, USA; application mode of the universal adhesive (etch-and-rinse, self-etch). The dependent variable was the enamel bond strength. The schematic presentation of the study design is shown in Figure 1. The details of the materials deployed in the present study are shown in Table 1.

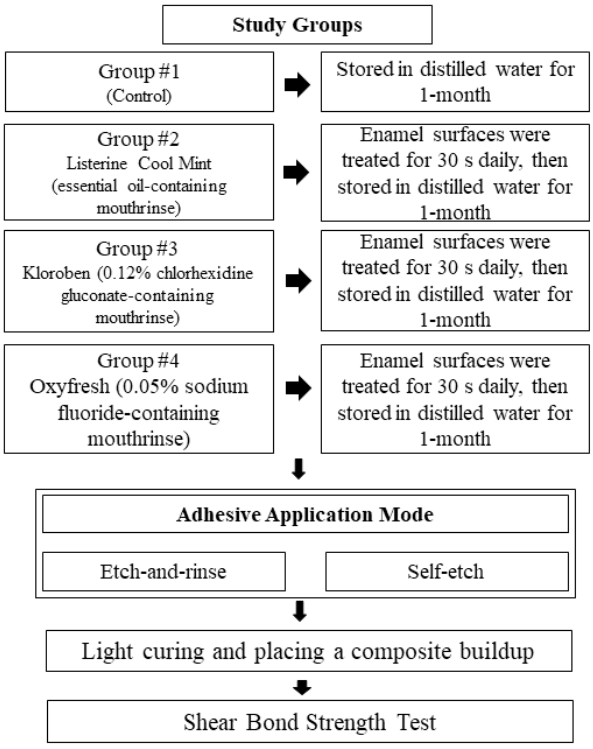

Fig. 1 – Schematic presentation of the study design.

Table 1

Materials used in the study

Material	Chemical composition
Single Bond Universal, 3M, Deutschland GmbH, Neuss, Germany	10-MDP, dimethacrylate resins, HEMA, Vitrebond copolymer, filler, ethanol, water, initiators, and silane.
Ultra-Etch (Ultradent)	35% phosphoric acid, water, cobalt aluminate blue spinel, glycol, and siloxane.
Dynamic Plus Universal Hybrid Composite, President Dental GmbH, München, Germany	Bis-GMA, TEGDMA, barium aluminosilicate and fumed silica fillers.
Listerine Cool Mint (essential oil-containing mouth rinse), Johnson & Johnson, New Jersey, USA	Thymol, eucalyptol, methyl salicylate, menthol, water, sorbitol solution, alcohol (30%), poloxamer 407, benzoic acid, mint and mint essences, sodium saccharin, sodium benzoate, green dye 3.
Kloroben (0.12% chlorhexidine gluconate-containing mouth rinse), Drogsan Pharmaceuticals, Ankara, Turkey	Chlorhexidine gluconate 0.12%, water, glycerin, ethanol, polysorbate 20, mint flavor aromatic composition, sodium saccharinate, FD & C, Blue 1.
Oxyfresh (0.05% sodium fluoride-containing mouth rinse), Oxyfresh Inc., Idaho, USA	Sodium fluoride, cetylpyridinium chloride, water, glycerin, propylene glycol, sorbitol, poloxamer 407, sodium chloride, potassium sorbate, sodium saccharin, citric acid, green dye, yellow dye.

10-MDP-10 – methacryloyloxydecyl dihydrogen phosphate; HEMA – 2-hydroxyethyl methacrylate; Bis-GMA – bisphenol A-glycidyl methacrylate; TEGDMA – triethylene glycol dimethacrylate.

Specimen preparation

The present *in vitro* study was conducted at Usak University. All operations were performed by the same person Approval from the Ethics Committee was not obtained as no human material was used in this study. In this study, 96 caries-free bovine incisor teeth were used. After removing all the soft tissues from the teeth with a scalar, the teeth were kept in 0.5% Chloramine-T at room temperature until they were used. For the preparation of flat enamel surfaces, individual teeth were embedded in acrylic resin with a silicone mold. After the acrylic resin was cured, the enamel surfaces were smoothed under water cooling with 400-grit silicon carbide (SiC) abrasive paper. The prepared teeth were then randomly distributed into four main groups of 24 teeth each according to the mouth rinse: Group I (Control – no mouth rinse was applied to the samples in the control group); Group II (essential oil-containing mouth rinse was applied to enamel surfaces of samples for 30 sec every day); Group III (0.12% chlorhexidine gluconate-containing mouth rinse was applied to the enamel surfaces of samples for 30 sec every day); Group IV (0.05% sodium fluoride-containing mouth rinse was applied to enamel surfaces of samples for 30 sec every day). Mouth rinses were applied to the prepared enamel surfaces daily for one month before bonding. The samples were kept in distilled water for a month. Storage solutions were changed weekly.

Shear bond strength testing

Before the application of the adhesive resin system, the enamel surfaces were polished with 600-grit SiC abrasive paper under water cooling in order to obtain clinically relevant and standardized smear layers on enamel surfaces. Samples in each group were randomly divided into two subgroups (n = 12) according to the universal adhesive application mode – the etch-and-rinse mode and the self-etch mode. For the etch-and-rinse mode, before applying the universal adhesive, the enamel surface was etched with 37% phosphoric acid gel for 15 sec, and the acid was rinsed for 30 sec and dried. Universal adhesive (Single Bond Universal, 3M Deutschland GmbH, Neuss, Germany) was applied to the acid-etched enamel surface according to the application instructions of the manufacturer. The adhesive was applied for 20 sec with active agitation and dried for 5 sec with gentle air pressure. The adhesive was polymerized with a LED light curing device (1,200 mW/cm^2, Elipar S10; 3M Unitek, Monrovia, CA, USA) for 10 sec. For the self-etch mode, the enamel surfaces were not pre-etched with phosphoric acid, and the universal adhesive was applied to the enamel surfaces, as previously explained.

After the adhesive application steps, the resin composite buildups were made using a silicone mold with a height of 4 mm and an internal diameter of 2 mm. Each layer of resin composite was polymerized for 20 sec with the same LED light-curing device. Bonded samples were kept in distilled water for 24 hrs and then subjected to an SBS test. The SBS tests were performed by the Instron universal testing machine (Instron 3220, Instron Corporation, Canton, MA) with a crosshead speed of 1 mm/min. The SBS was expressed as megapascals (MPa) by dividing the maximum force value (Newton) by the bonding area (mm^2).

After the SBS test, the debonded surfaces were evaluated under x20 magnification with a stereomicroscope (Meade Bresser Biolux, Meade Bresser, Rhede, Germany), and failure modes were determined. Failure modes were classified as follows: (1) “adhesive failure” – if the debonding occurred in more than 80% of adhesive; (2) “cohesive failure” – if the debonding occurred in one of the substrates (enamel or resin composite) in more than 80% of adhesive; (3) “mixed failure” – with a combination of adhesive and cohesive failure.

Statistical analysis

A two-way Analysis of Variance (ANOVA) and Tukey's HSD test (p = 0.05) were used in analyzing SBS data. Factors were mouth rinse (Listerine Cool Mint/Chlorhexidine mouth rinse/fluoride mouth rinse) and adhesive application mode (etch-and-rinse/self-etch). A two-way ANOVA, along with Tukey's HSD test, was applied together with each application mode (p = 0.05). All statistics were made with the SPSS version 12 software (SPSS, Chicago, IL, USA).

Results

The SBS mean values and standard deviations, failure mode distributions for adhesive application modes, and mouth rinses were summarized in Table 2. Likewise, a bar graph shows the SBS of the mouth rinses groups concerning adhesive application modes in Figure 2. Two-way ANOVA revealed that there was no statistically significant interaction between the effects of mouth rinse and adhesive application mode on SBS (p = 0.971). However, it showed that only adhesive application mode significantly affected SBS (p < 0.05) and not mouth rinse (p = 0.434). Application of universal adhesive in etch-and-rinse mode showed significantly higher SBS than those in self-etch mode regardless of mouth rinse. Predominance failure modes were cohesive and mix failures for all etch-and-rinse groups, while predominance failure modes were adhesive and mix failures for all self-etch groups.

Discussion

Antibacterial mouth rinses are mostly used by patients with high caries risk in order to reduce the cariogenicity of dental plaque since it is difficult to completely clean the dental plaque, which is the primary factor of dental caries, and

Table 2

Shear bond strength (SBS) and distribution of failure types for all groups (n = 12)

	Adhesive application mode			
	etch-and-rinse		self-etch	
Groups	SBS	failure mode	SBS	failure mode
I	33.79 ± 5.9 $^{a\,A}$	C > M > A	23.85 ± 7.4 $^{a\,A}$	A > M > C
II	32.53 ± 4.3 $^{a\,A}$	C > M > A	21.87 ± 5.9 $^{a\,B}$	A > M > C
III	37.51 ± 5.8 $^{a\,A}$	C > M = A	27.27 ± 6.1 $^{a\,B}$	A = M > C
IV	35.52 ± 5.2 $^{a\,A}$	C > M > A	21.38 ± 5.4 $^{a\,B}$	A > M > C

A – adhesive failure; M – mixed failure; C – cohesive failure. Group I – distilled water; Group II – Listerine Cool Mint (essential oil mouth rinse); Group III – Kloroben (0.12 chlorhexidine gluconate mouth rinse); Group IV – Oxyfresh (0.05% sodium fluoride mouth rinse). Results of SBS test are given as mean ± standard deviation. Different lowercase superscripts represent the significant difference in the same column (p < 0.05). Different uppercase superscripts represent the significant difference in the same row (p < 0.05).

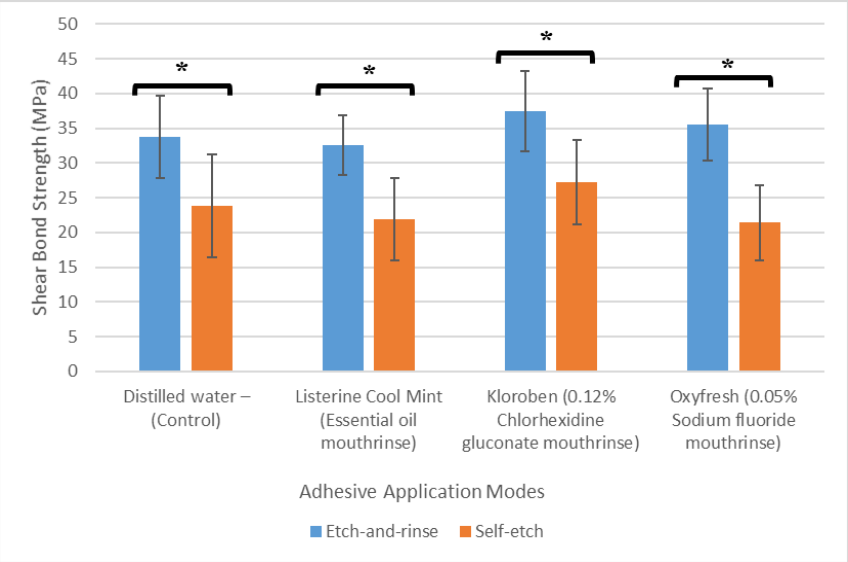

Fig. 2 – Bar graph depicting shear bond strength of the tested groups concerning adhesive application modes. Values are expressed in megapascals (MPa). *Statistically significant difference between groups analyzed using two-way ANOVA (p < 0.05).

prevent its recurrence. However, there is not enough information in the literature about how mouth rinses affect the enamel bonding of universal adhesives. Therefore, the present study evaluated the effects of three different mouth rinses, including an essential oil-containing mouth rinse, on the enamel bond strength of a universal adhesive applied in different modes.

Bovine teeth were used instead of human teeth in the SBS test in the present study. It has been shown that bovine teeth can replace human teeth in both enamel and dentin bond strength tests [13]. It has been reported that bovine teeth have a mineral distribution similar to human enamel [14]. One advantage of using bovine teeth is obtaining large numbers of teeth from animals of similar age groups and with similar diets in a short time, which enables the age and chemical structures of the teeth to be standardized. Because of these advantages, bovine teeth were preferred in the present study as an alternative to human teeth.

In the present study, it was noticed that enamel bonding was significantly affected by the application mode of the adhesive regardless of mouth rinses. Since the universal adhesive system used in this study is an adhesive system – Single Bond Universal (3M ESPE, USA), which has been used in many studies in the literature – it was deliberately chosen in order to understand whether the findings of the present study correlated with the literature. In previous studies, it has been reported that the application of Single Bond Universal adhesive to enamel in the etch-and-rinse mode provides significantly higher bonding strengths compared to the self-etch mode [15–17]. In this respect, the findings of the present study are compatible with the literature.

Etching the enamel surface with a separate acid agent before the application of the universal adhesive, in other words, applying the universal adhesive in the etch-and-rinse mode, has been shown to increase the enamel bonding due to the increase in the porosity on the enamel surface and the infiltration of the resin into these porosities [15]. During the acid etching process, there is a loss of material at a depth of 10 μm from the enamel surface, and a porous layer of 5–50 μm is formed [15, 18]. Polymerization of resin after infiltrating into this porous structure allows the composite to adhere micromechanically to the enamel surface.

The two-way ANOVA revealed no significant interaction between factors types of mouth rinse and adhesive application modes. That may explain the findings of the study. It was observed that the application of mouth rinses tested on enamel surfaces for 30 sec daily for a month did not affect the enamel bonding even when applying the universal adhesive used in the study with two different application modes. In previous studies, it has been reported that mouth rinses containing fluoride cause the formation of fluorapatite crystals on the enamel surface and increase the number of minerals on the enamel surface [19]. Similar to the findings in our study, it was reported in the study of Elzuhery et al. [19] that the fluoride-containing mouth rinse did not affect the enamel bonding of the adhesive systems. Although the mouth rinse containing fluoride increases the number of minerals on the enamel surface, this layer is removed by grinding during the smear layer formation process before the application of the adhesive. Therefore, the presence of this mineral-dense layer may not have affected the enamel bonding of the adhesive in both application modes.

In the failure type analysis, cohesive failure type was predominant in etch-and-rinse groups regardless of mouth rinse. However, adhesive and mix failure types were predominant in self-etch groups regardless of mouth rinse. It can be anticipated that cohesive would correspond to higher SBS values in etch-and-rinse groups or *vice versa* in self-etch groups [20].

Similarly, the reason why the bonding of the tested adhesive was not affected by chlorhexidine and Listerine-containing mouth rinses might be because the layers on the enamel surface affected by these mouth rinses were removed during the grinding prior to adhesive bonding. A study supporting this finding was conducted by Demir et al. [21], where it was reported that the mouth rinses containing chlorhexidine gluconate applied daily for 30 sec for two weeks did not affect the enamel bonding of the orthodontic composite.

Conclusion

Pre-etching of the enamel surface with phosphoric acid before the application of universal adhesive to enamel surfaces on which mouth rinses containing essential oil, chlorhexidine, or fluoride have been applied can significantly increase the bonding strength of the adhesive. On the other hand, it was determined that the mouth rinses tested in the study did not affect the adhesion of the universal adhesive to the enamel negatively in the self-etch mode. For this reason, no waiting period may be required to ensure the bonding quality of the resin-enamel interface in patients using mouth rinses tested in this study prior to the treatment of dental caries using resin adhesive systems.

Conflict of interest

The author declares no conflict of interest.

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Surgical treatment of parathyroid cysts: case series and review of literature

Hirurško lečenje paratiroidnih cisti: serija bolesnika i pregled literature

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Abstract

Introduction. Parathyroid cysts (PCs) are divided into two categories: functional and nonfunctional. If large enough, both types of PCs can present as a mediastinal or cervical mass in 1–5% of patients. **Case report.** A retrospective analysis of the data on patients operated on for primary hyperparathyroidism or cervical/mediastinal mass from 2016 to 2021 was conducted. An analysis of the demographic data of the patients, data on preoperative fine needle aspiration biopsy, level of parathyroid hormone in serum pre- and postoperatively, level of serum calcium, as well as on clinical presentation of the disease, was carried out. In this five-year period, a total of 555 patients were operated on, in whom the parathyroid gland was described as a definitive pathohistological finding. Of the total number, PCs were found in seven cases. In five out of the seven cases, PC was nonfunctional. Four female and three male patients were operated on due to PC. The mean age of operated patients was 49.8 years. In one patient, the nonfunctional cyst was represented as a cervical and upper mediastinal mass with a maximal diameter of 10 cm. **Conclusion.** Although PCs represent about 0.5% of all changes in the parathyroid glands, they can be suspected preoperatively, especially if a water-like liquid is obtained by a fine needle aspiration biopsy. In order to remove the PC completely without making a lesion on the capsule, with the aim of avoiding parathyreomatosis, but to preserve the recurrent laryngeal nerves, the operation should be performed by an experienced endocrine surgeon.

Key words:
diagnosis, differential; parathyroid glands; surgical procedures, operative.

Apstrakt

Uvod. Paratiroidne ciste (PC) mogu se podeliti u dve kategorije: funkcionalne i nefunkcionalne. Oba tipa PC, ukoliko su dovoljno velika, mogu se prezentovati kao medijastinalna ili cervikalna masa kod 1–5% bolesnika. **Prikaz bolesnika.** Urađena je retrospektivna analiza podataka bolesnika operisanih u periodu od 2016. do 2021. godine zbog primarnog hiperparatiroidizma ili postojanja cervikalne/medijastinalne mase. Sprovedena je analiza demografskih podataka bolesnika, podataka o preoperativnoj aspiracionoj biopsiji, nivou paratiroidnog hormona u serumu pre i postoperativno, nivou serumskog kalcijuma, kao i o kliničkoj prezentaciji bolesti. U petogodišnjem periodu operisano je ukupno 555 bolesnika, kod kojih je patohistološkim nalazom potvrđeno postojanje patološki izmenjene paratiroidne žlezde. Od ukupnog broja, kod njih sedam opisana je PC. Kod pet od ovih sedam bolesnika, PC je bila nefunkcionalna. Četiri osobe ženskog pola i tri osobe muškog pola operisane su zbog PC. Prosečna starost operisanih bolesnika iznosila je 49,8 godina. Kod jednog bolesnika nefunkcionalna PC se manifestovala kao cervikalna masa u gornjem medijastinumu, maksimalnog promera od 10 cm. **Zaključak.** Iako PC predstavljaju oko 0,5% svih promena na paratiroidnim žlezdama, na njih se može posumnjati preoperativno, naročito ako se aspiracionom biopsijom tankom iglom dobije tečnost slična vodi. Da bi se PC odstranila u celosti i bez lezije kapsule, sa ciljem da se izbegne paratireomatoza, kao i da se sačuvaju rekurentni laringealni nervi, operaciju bi trebalo da uradi iskusan endokrini hirurg.

Ključne reči:
dijagnoza, diferencijalna; paratireoidne žlezde; hirurgija, operativne procedure.

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Introduction

Cysts of the parathyroid gland or parathyroid cysts (PCs) are relatively rare and represent 0.5% of all parathyroid pathology [1]. Depending on hormonal secretion, PCs can be divided into two categories: functional and nonfunctional. Functional PCs present with symptoms of primary hyperparathyroidism. Both functional and nonfunctional PCs can be presented as anterior n or mediastinal mass with symptoms of compression of surrounding organs (dysphagia, trouble with breathing). Until now, around 360 PCs have been published in the literature as case reports or case series. In this paper, we described seven patients with PCs [2].

Case report

We conducted a single-center study with a retrospective analysis of medical records of operated patients from January 2016 to June 2021. We identified all patients in whom pathohistological (PH) findings detected PCs after surgical treatment. PH PCs were defined with a thin wall composed of cuboid cells. The study was conducted following the Good Clinical Practices with their origins in The Declaration of Helsinki. We collected informed consent from all patients included in the study. We reviewed the demographic data of the patients, data on preoperative fine needle aspiration biopsy (FNAB), level of parathyroid hormone (PTH) in serum pre- and postoperatively, level of serum calcium, and clinical presentation of disease (difficulty swallowing, neck or mediastinal mass).

Over the five years, we reviewed a total of 555 patients operated on due to primary hyperparathyroidism, neck or mediastinal mass with PH confirmation of PC after the operation. We excluded patients who were operated on due to secondary or tertiary hyperparathyroidism. Of 555 operated patients, in seven cases, the PH onset was PC. Table 1 shows relevant clinical characteristics (age, gender, presentation of disease – mediastinal or neck mass, functional/nonfunctional cysts), PTH, calcium, and phosphate levels of these patients. All patients had neck discomfort and were presented with either cervical or mediastinal mass, which was one of the indications for the operation.

Four female and three male patients were operated on. The mean age of operated patients was 47.8 years at the time of the operation, ranging from 38 to 64 years. Two of the patients had functional PCs with clinical manifestations (bone pain, nephrocalcinosis) of primary hyperparathyroidism with confirmed high levels of calcium and PTH level. In the remaining patients, serum PTH and calcium levels were in the range.

In five of our patients, a preoperative FNAB was performed, and water-like fluid was aspirated. In the aspirated material, the high PTH level was detected, and in all four cases, it was significantly high, ranging from 311 to 2,500 ng/L. Cytology was also performed; the pathological finding was typical for cysts: liquid with no cells found.

Case number two was the youngest patient, a male in whom a nonfunctional PC was presented as a large cervical-partially mediastinal mass. Computed tomography (CT) was performed, and a cyst with a maximal diameter of 10 cm was seen (Figure 1). That was the largest PC in our study (Figure 2). PH finding was similar in all cases: thin wall cyst with one layer of cuboid cells. Immunohistochemistry was positive for PTH, while thyroid transcription factor 1 (TTF1) was negative. PH finding of the thin cyst wall (of the patient under ordinal number 2) is shown in Figure 3.

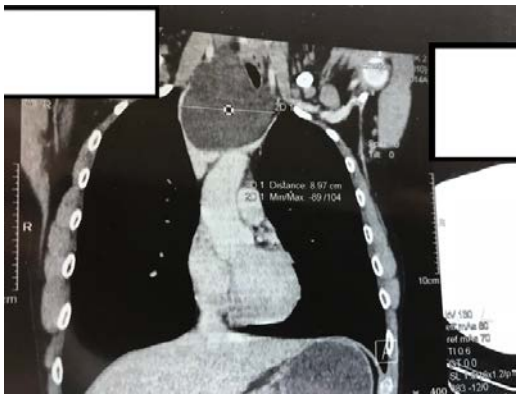

Fig. 1 – Computer tomography of the neck shows a large mass in the neck and partially in the upper mediastinum in the second patient.

Table 1

Clinical characteristics of patients with parathyroid cyst

Patient number	Gender	Age (years)	Type of cyst	Biggest diameter of the cyst (cm)	Serum PTH (ng/L) Pre/Post-operative	PTH in aspiration (ng/L)	Serum Ca/PO_4 (mmol/mL)	Location of gland
1	F	52	functional	3.5	78 /18	-	2.62/1.2	dex. inf.
2	M	38	nonfunctional	10	37.8	340	2.42/1.1	dex. inf.
3	M	64	functional	2.9	131/7	-	2.46/0.86	sin. inf.
4	M	64	nonfunctional	4.2	37/-	311	2.23/0.95	dex. inf.
5	F	47	nonfunctional	3.3	42/31	2,500	2.24/1.04	sin. inf.
6	F	36	nonfunctional	2.9	36/-	921	2.31/-	sin. inf.
7	F	48	nonfunctional	3.35	44/-	890	2.28/-	sin. inf.

F – female; M – male; PTH – parathyroid hormone; Ca – calcium; PO_4 – phosphate; dex. inf. – dexter inferior; sin. inf – sinister inferior.
Reference ranges for serum PTH, Ca and PO_4 are 15-65 ng/L, 2.15–2.65 mmol/mL and 0.8–1.55 mmol/mL, respectively.

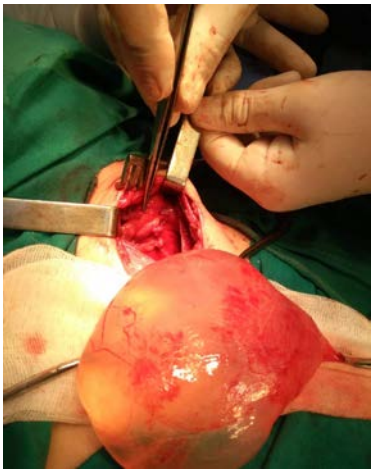

Fig. 2 – Intraoperative finding of big parathyroid cyst in the second case.

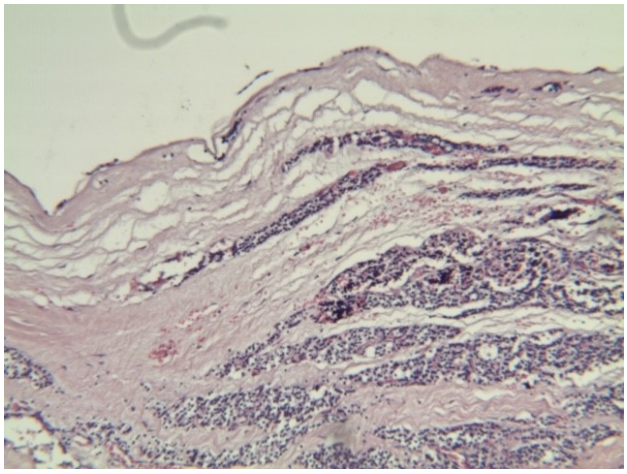

Fig. 3 – Histopathological finding of the thin cyst wall (hematoxylin and eosin, × 100) of the second patient.

Discussion

The first description of the parathyroid gland was made by Sir Richard Owen, who described the parathyroid gland in an Indian rhino [3]. A few decades later, in the nineteenth century, Sandström et al. [4] first described the parathyroid gland in humans. Since then, around 360 PCs have been described in literature [2]. Since parathyroid glands arise from the third and fourth branchial pouches, they can be located anywhere from the floor of the primitive foregut to its final location. Large PCs frequently descend into the mediastinum due to a combination of gravitation and the effect of negative intrathoracic pressure [5].

PCs can be divided into two categories: functional and nonfunctional. Diagnoses for both types of PCs are made preoperatively based on serum PTH and calcium levels [1]. In functional PC, serum PTH and calcium levels are elevated, while phosphate level is lower or between range levels. Scintigraphy is indicated when a suspicious PC is noticed. In functional PC, scintigraphy is positive, while in nonfunctional PC, it is negative [6]. Besides PTH values, localization and relation to other vital organs should be set. For that purpose, an ultrasound of the neck is usually enough; sometimes, CT or magnetic resonance imaging is needed due to large and especially mediastinal cysts [7]. When a hypoechoic nodule is seen on ultrasound, FNAB should be done and sent for the detection of the PTH level. Liquid from PCs is usually water-like, unlike the liquid from thyroid cysts, which is usually yellowish or dark brown. The color and PTH value of punctuated content is greatly valued when the PC is intrathyroidal [8].

In 2018, Papavramidis et al. [2] conducted a meta-analysis from 1905 until 2016 and found 359 reported cases of PCs described in 218 articles. The mean age of the population was 49.24 years, and the mean age of our patients was 47.8 years. The ratio between men and women was 1:1.85. Female predominance is seen in the reported meta-analysis. In our study, female to male ratio was 1:1.3. The most frequent symptom described in Papavramdisis [2] meta-analysis was neck mass, followed by compressive symptoms and parathyroid dysfunction. In two of our patients, PC was functional, followed by high levels of serum calcium. In the remaining five cases, PCs were detected as cervical masses. Preoperative FNAB was performed in five of our patients, in which water-like fluid was removed, and high levels of PTH were detected; cytology findings showed noncellular content.

The mean diameter of PCs was 4.81 ± 2.88 cm, ranging from 0.5 to 15 cm. Males usually had bigger PCs than females. Our second patient had a PC that was 10 cm in diameter. As far as the location is concerned, bigger PCs were located in the middle compartment of the mediastinum.

DeQuervain et al. [9] first operated on a patient with mediastinal PC in 1925. Mediastinal PCs are usually asymptomatic; they usually show up incidentally on a chest X-ray. As they grow larger, dyspnea and dysphagia may occur. Hoarseness due to paralysis of the recurrent laryngeal nerve has been less frequently reported [10].

Treatment of PCs can be either surgery (which is most common) or sclerosing therapy. Sclerosing therapy can be used as an alternative if there are contraindications for surgery. It is performed with ethanol or tetracycline less frequently. This way of treatment may be associated with many complications, such as neck pain, neurotoxicity, and recurrent nerve palsy [11]. All functional PCs are best treated by surgical excision. Nonfunctional PCs sufficiently large to be symptomatic or obstructing should also be surgically removed [12].

Conclusion

Although PCs represent about 0.5% of all parathyroid pathology, they can be diagnosed preoperatively, especial-ly if a water-like liquid is found with FNAB. Experienced endocrine surgeons should perform the operation by preserving the cyst wall to avoid parathyreomatosis and recurrent laryngeal nerve preservation.

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CASE REPORT
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Above-knee amputation due to necrotizing fasciitis caused by a gas-producing strain of *Escherichia coli* and negative pressure therapy assisted closure of a large open wound

Natkolena amputacija zbog nekrotizujućeg fasciitisa izazvanog sojem *Escherichia coli* koji produkuje gas i zatvaranje velike otvorene rane uz pomoć terapije negativnim pritiskom

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Abstract

Introduction. Nonclostridial gas-forming soft tissue infections (NGSTI) are rare, rapid progressive infections characterized by high mortality and high amputation rates. Surgical debridement is crucial in therapy, and it results in complex wounds that need to be closed in order to prevent secondary morbidity. **Case report.** Herein we present a case of NGSTI in a 68-year-old diabetic patient with acute thrombosis of popliteal artery aneurysm and radiological signs of gas in his right leg and the urinary bladder wall. The infection was caused by a gas-forming strain of *Escherichia coli*. In the early stage of the disease, the patient was treated with antibiotics and femoral amputation. A vacuum-assisted closure (VAC) treatment was applied to close the amputation wound. Administered VAC therapy resulted in primary wound closure without complications 17 days after surgery. **Conclusion.** Negative pressure increases the clearance of fluid and infection from the wound but also increases wound contraction and approximation of skin flaps. To avoid extensive reconstructive surgery, VAC therapy can be a good adjunctive treatment for closing large open wounds in patients with NGSTI.

Key words:
amputation, surgical; diabetes mellitus; escherichia coli; necrotizing fasciitis; wound closure techniques.

Apstrakt

Uvod. Neklostridijalne infekcije mekih tkiva sa pisustvom gasa (NIMTPG) su retke, rapidno progresivne infekcije, koje se karakterišu visokim mortalitetom i visokom stopom amputacija. U terapiji je ključan hirurški debridman, posle koga ostaju kompleksne rane, koje je potrebno zatvoriti da bi se sprečio sekundarni morbiditet. **Prikaz bolesnika.** Prikazan je bolesnik sa NIMTPG, dijabetičar, star 68 godina, sa akutnom trombozom aneurizme poplitealne arterije i radiološkim znacima gasa u desnoj nozi i zidu mokraćne bešike. Infekcija je bila izazvana sojem *Escherichia coli* koji produkuje gas. Bolesnik je u u ranoj fazi lečen antibioticima i amputiran mu je femur. Za zatvaranje amputacione rane primenjeno je vakumom-asistirano zatvaranje (VAZ). Primenjena terapija je dovela do primarnog zatvaranja rane bez komplikacija, 17 dana posle operacije. **Zaključak.** Negativan pritisak povećava klirens tečnosti i infekcije iz rane, povećava i pritisak u rani, kao i približavanje kožnih „flapova“. Terapija VAZ može biti dobar pomoćni tretman za zatvaranje velikih rana kod bolesnika sa NIMTPG u cilju izbegavanja velikih rekonstruktivnih zahvata.

Ključne reči:
amputacija, hirurška; dijabetes melitus; escherichia coli; fasciitis, nekrotizujući; rana, zatvaranje, tehnike.

Introduction

Nonclostridial gas-forming soft tissue infections (NGSTI) are characterized by fulminate widespread necrotizing changes of any soft tissue compartment, production of gas, and systemic toxicity. The disease has an aggressively invasive course, frequent loss of extremities, and mortality as high as 42.9–64.5% [1, 2]. Incidence of severe necrotizing soft tissue infections caused by all known microorganisms has been estimated between 0.4 and 0.53 cases *per* 100,000 people, and only a handful of them are characterized as gas-forming [1, 3, 4]. It is a relatively rare entity and can appear after trauma, surgical intervention, minor injury, and sometimes even spontaneously [3].

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The infection is usually caused by gram-positive cocci, gram-negative rods, or a combination of microorganisms. Of all affected patients, it is most common in those with diabetes mellitus (DM). Most often, it has a short incubation time with sudden onset of pain, development of crepitus, and soft tissue induration; discoloration may also be present. Plain X-rays identify gas in deep tissues, and computed tomography (CT) imaging or magnetic resonance imaging may assess the spreading of infection within muscles. Signs of intoxication can develop rapidly, and many patients present with septic shock at the time of their admission [3, 5].

Treatment consists of prompt initiation of antimicrobial therapy, which consists of broad-spectrum antibiotics until a causative agent is identified, intensive care support, and wide surgical debridement. Delay of surgical treatment is associated with higher morbidity and mortality. Usually, amputation is necessary to control infection, whereas functional limb salvage is rare [5]. After such wide surgical debridement, large open wounds persist. The prolonged open wound period induces wound skin marginal contraction and inversion. To close such wounds, complex surgical interventions are needed and delay in coverage may result in infection and secondary morbidity (suboptimal stump function, deformation, infection of skin graft donor site, necrosis of free flap).

To facilitate and accelerate the closure of open wounds, negative pressure therapy [vacuum-assisted closure (VAC) technique] is described in literature [6, 7]. Positive effects of the VAC technique on open wounds are control of tissue edema by removing tissue fluid, preventing bacterial and fungal colonization, tissue angioneogenesis, and enhancing granulation tissue growth. Although VAC therapy can improve the condition of a wound, it cannot close it completely; therefore, other operations are required for definitive wound closure [6, 8, 9].

Case report

A 68-year-old male with a previous history of DM presented to the Emergency Department with a history of pain in his right lower limb for the past five days. The patient did not have any history of preceding trauma. The pain was dull, aching, with high intensity, and non-radiating. He was not aware of his diabetes status.

On physical examination, he was afebrile, with a pulse of 100 bpm, his blood pressure was 180/100 mmHg, and he had dyspnea. Locally, his right leg was generally swollen and tender. He had local areas of erythema on the medial aspect of his right thigh and the ventral side of his knee. In the pretibial area, skin discoloration was present with crepitations, but also with areas of fluctuation. The popliteal, posterior tibial, and *dorsalis pedis* pulsations were absent. The foot was cold, but there were no ulcers or gangrene signs over the foot.

Laboratory analysis showed red blood cells count of 3.46×10^{12}/L [reference range (RR) $4.44–5.6 \times 10^{12}$/L], white blood cells count (WBC) of 15.14×10^{9}/L (RR $3.91–10.0 \times 10^{9}$/L), granulocytes count of 12.95×10^{9}/L (RR $1.8–6.9 \times 10^{9}$/L), platelet count of 241×10^{9}/L (RR $166–308 \times 10^{9}$/L), hemoglobin level of 84 g/L (RR 135–169 g/L), and random blood glucose level of 16.9 mmol/L (RR 3.9–6.1 mmol/L). His blood urea was 11.1 mmol/L (RR 3.0–9.2 mmol/L), creatinin 102 μmol/L (RR 62–115 μmol/L), creatinine kinase 8.445 U/L (RR 30–200 U/L), aspartate-aminotransferase 460 U/L (RR 5–34 U/L), alanine-aminotransferase 254 U/L (RR 0–55 U/L), albumin level was 24 g/dL (RR 32–46 g/L), D-dimer 3.348 ng/mL (normal values < 198 ng/mL), sodium level 132 mmol/L (RR 136–146 mmol/L), and potassium level was 3.7 mmol/L (RR 3.5–5.1 mmol/L). Of inflammatory markers, he had C-reactive protein (CRP) of 303.3 mg/L (RR 0.0–5.0 mg/L), procalcitonin of 1.97 ng/mL (normal values < 0.05 ng/mL), and fibrinogen of 8.25 g/dL (RR 2.10–4.0 g/dL).

CT scan of his right lower extremity showed the presence of gas inclusions in the region of his plantar fascia, muscles of the calf, popliteal *fossa*, and medial aspect of his thigh up to his hip. There were also gas inclusions in the muscular layer of the urinary bladder wall. The scan also showed a thrombosed popliteal aneurysm diameter of 60 mm, with no flow distally (Figure 1).

Because of extensive infection and muscle necrosis with inclusions of gas and ischemia, the patient underwent

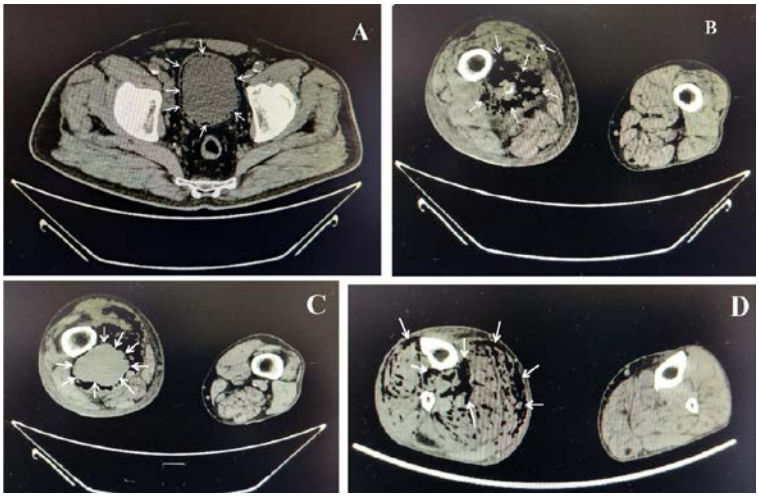

Fig. 1 − Pelvis and lower extremities computed tomography scan: A) Gas inclusions in urinary bladder wall (arrows); B) Gas inclusions in muscles of the right thigh; C) Right popliteal artery aneurysm (arrows) with gas inclusion around it; D) Gas inclusions in muscles of the right calf (arrows).

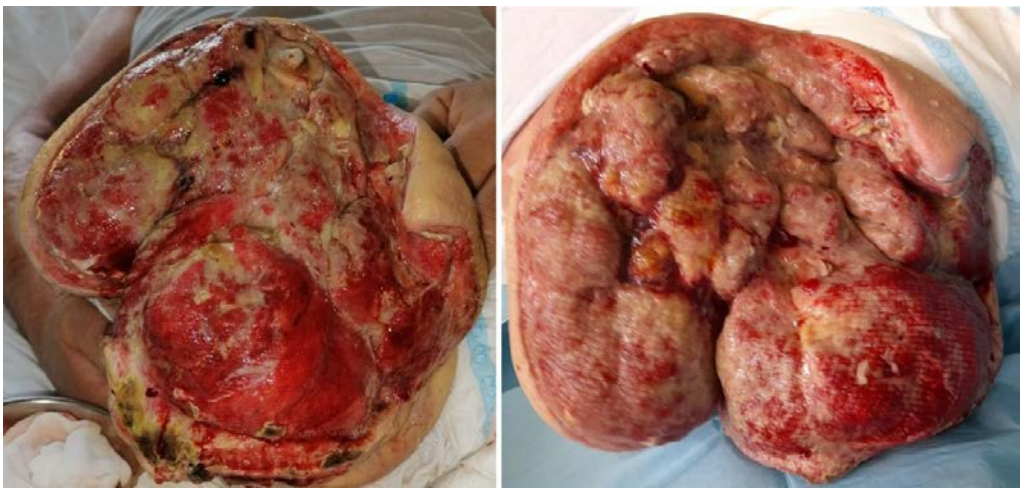

Fig. 2 – Postamputation wound on the first (left) and fourth (right) postoperative day.

right above-knee amputation with an incision on the medial aspect of the proximal third of the right thigh. Intravenous penicillin G combined with clindamycin was administered for two days until the necrotic tissue and urine culture showed the growth of *Escherichia coli (E. coli)*. According to the antibiogram, the patient was on piperacillin-tazobactam therapy. Furthermore, tissue swabs and samples were taken for testing on anaerobic agents, but the results were negative. On the day when antibiotic therapy was changed, inflammatory marker values were as follows: CRP 194.6 mg/L and procalcitonin 1.05 ng/mL.

In response to intensive therapy, the patient showed a significant improvement postoperatively. On the fifth day after admission, a control CT scan was made, which showed no signs of gas in the urinary bladder wall. On the eighth day, the wound swab was sterile (Figure 2). After ten days of thorough wound debridement and irrigation, the patient underwent two cycles of VAC therapy for wound closure. VAC system was placed over the patient in analgosedation. The wound volume had decreased prominently, and a retraction of muscles had been achieved. We were able to close the wound without tension 17 days after the treatment without infection. The patient was discharged without complications 22 days after admission. The patient was followed up regularly at the outpatient department and without complications.

Discussion

NGSTI is quite a rare, life-threatening condition, and diagnosis is based on clinical findings, radiological examinations, and microbiological investigations [3]. Most of these patients have underlying diseases such as DM or peripheral atherosclerotic vascular diseases [8–10]. However, to the authors' knowledge, there are very few reports on patients with such conditions and concurrent peripheral artery aneurysms or acute ischemia [11]. Likewise, in the available literature, it is rare for *E. coli* to produce gas and severe deep soft tissue infection [12, 13].

In the presented case, multiple tissue sample cultures showed the growth of *E. coli* as the monomicrobial causative agent. Gas inclusions were present in the lower limb and the muscle layer of the urinary bladder wall. A possible explanation for gas in the urinary bladder wall is that this was the primary site of infection with a facultative anaerobic strain of *E. coli*, which was in the surrounding of glucosuria [14], and that it was secondarily transferred to a skin defect on the right lower limb. Moreover, due to a thrombosed popliteal artery, limb ischemia further contributed to bacterial growth.

The most important in therapy are surgical intervention, antibiotic therapy, and intensive care support [3, 15]. Surgical intervention consists of surgical debridement of all necrotic tissue. In many cases, NGSTI surgical treatment of involved extremities consists of amputation and/or fasciotomy. Amputation might be beneficial in cases where compromised neurovascular supply exists and when attempts to salvage the involved limb may lead to metabolic overload and secondary organ failure [5, 16]. In this infective setting, an open stump wound is necessary for wound debridement and infection clearance. However, this open surface results in exposing muscles and soft tissues with wound marginal skin contraction and inversion, thus aggravating the approximation of wound skin flaps. This condition diminishes the possibility of later wound closure.

In this case, there were clinical and radiological signs of widespread infection and, simultaneously, signs of acute critical limb-threatening ischemia because of a thrombosed popliteal artery aneurysm. Since then, reconstructive vascular surgery treatment was associated with extremely high risk in this concurrent infective and ischemic settings. It has been decided that the above-knee amputation be done as a life-saving procedure without prior fasciotomy.

Lee et al. [6] showed that the restoration of the tissue pressure provided by fascia and skin is the key to large open wound closure. Following the same principle, we decided to apply the VAC system on the postamputation stump. We achieved it by fixating a sponge on the muscle fascia layer to achieve the negative pressure on muscles while preserving marginal skin and subcutaneous tissue. The VAC therapy suction pressure was set at 100 mmHg to increase tissue pressure and wound fluid removal while

maximizing wound contraction and microvascular blood flow [6, 17]. Centripetal compression effect enhanced approximation of skin flaps.

In our patient, there was no skin necrosis, and the administration of negative pressure therapy facilitated primary wound closure. For successful contouring of wound surface area, an appropriate wound preparation was vital for successful closure. Negative pressure was acting twofold; it was involved in wound bed preparation and infection clearance.

Hyperbaric oxygenation as an adjunct treatment [3, 15, 18] has been discussed, but it has been decided not to be used because of clinical and radiological signs of good response to previous therapeutic modalities and in order to accelerate closure of stump wounds by administering negative pressure therapy.

Conclusion

We presented a rare case of necrotizing fasciitis with gas inclusions caused by a gas-producing strain of *E. coli* with concurrent thrombosed popliteal aneurysm and acute limb ischemia, which was treated with above-knee amputation and modulated negative pressure therapy for closure of the large open wound. Negative pressure therapy decreases the open wound area and minimizes the necessity for secondary operation after treatment of NGSTI.

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CASE REPORT

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What can hide an enlarged lymph node of a patient with prostatic adenocarcinoma?

Šta može da sakrije uvećan limfni čvor kod bolesnika sa adenokarcinomom prostate?

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Abstract

Introduction. Adenocarcinoma is the most common prostatic malignancy, where clinical management, the Gleason score, and recent updates in prostate cancer staging play critical roles. Mantle cell lymphoma (MCL) originates from the malignant transformation of B lymphocyte in the outer edge of the lymph node follicle, with pathognomonic overexpression of cyclin D1. We present a rare case of two simultaneous neoplasms occurring in the same patient. **Case report.** During the hospital preoperative examinations in a 68-year-old patient planned for radical prostatectomy, using multislice computed tomography, a tumor mass confined to the prostate, but also excessive lymph node enlargement, was revealed. Tissue specimens were analyzed after the hematoxylin and eosin staining was performed, as well as an immunohistochemical (IH) biomarker panel. Having performed a thorough histological examination, a diagnosis of prostatic adenocarcinoma was made, with a Gleason score 3 + 4 = 7 and Grade Group 2 of the International Society of Urological Pathology (ISUP). Microscopic analysis of lymph node involvement showed unexpected, diffuse proliferation of small lymphoid cells with irregular nuclei, wide mantle zone, and hyalinized blood vessels. After using IH staining for specific markers, another diagnosis was set, and it was non-Hodgkin MCL. **Conclusion.** A prostatic adenocarcinoma can rarely coexist with an undiagnosed lymphoproliferative disease, such as non-Hodgkin MCL in our case.

Key words:
adenocarcinoma; comorbidity; diagnosis; immunohistochemistry; lymphoma, mantle-cell; prostatic neoplasms.

Apstrakt

Uvod. Adenokarcinom je najučestaliji malignitet prostate, kod koga kritičnu ulogu igraju klinički pristup, *Gleason* skor i obnovljeni i unapređeni načini određivanja stadijuma karcinoma prostate. Limfom *mantle* ćelija (LMĆ) potiče od maligno transformisanih B limfocita spoljašnje zone folikula limfnog čvora, sa patognomoničnom ekspresijom ciklina D1. Prikazan je bolesnik sa retkom istovremenom pojavom dve neoplazme. **Prikaz bolesnika.** Tokom bolničkih preoperativnih pregleda kod bolesnika starog 68 godina, planiranog za radikalnu prostatektomiju, korišćenjem multislajsne kompjuterske tomografije, otkrivena je tumorska masa ograničena na prostatu, ali i značajno uvećanje limfnih čvorova. Uzori tkiva analizirani su posle hematoksilin eozin bojenja i imunohistohemijskog (IH) obeležavanja odgovarajućeg panela markera. Posle histološke procene, postavljena je dijagnoza adenokarcinoma prostate, sa *Gleason* skorom 3 + 4 = 7 i gradusom grupe dva prema *International Society of Urological Pathology* (ISUP). Mikroskopskom analizom regionalnih limfnih čvorova utvrđena je neočekivana difuzna proliferacija malih limfoidnih ćelija sa nepravilnim jedrima, širokom *mantle* zonom i hijalinizovanim krvnim sudovima. Primenom IH bojenja u cilju detekcije specifičnih makera postavljena je i dijagnoza *non-Hodgkin*-ovog LMĆ. **Zaključak.** Adenokarcinom prostate veoma retko može koegzistirati sa prethodno nedijagnostikovanim limfoproliferativnim oboljenjem, kao što je u ovom slučaju *non-Hodgkin*-ov LMĆ.

Ključne reči:
adenokarcinom; komorbiditet; dijagnoza; imunohistohemija; limfom, mantle-ćelijski; prostata, neoplazme.

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Introduction

Adenocarcinoma is the most common malignancy of the prostate. Clinical management, the Gleason score, and the World Health Organization/International Society of Urological Pathology (WHO/ISUP) grading system play a critical role in predicting biological behavior and prognosis of adenocarcinoma. Radical prostatectomy with dissection of pelvic lymph nodes (LNs) is a routine procedure in patients with high serum prostate-specific antigen (PSA) level or high combined Gleason score (≥ 8) performed in order to prevent metastatic disease [1]. Due to the high sensitivity of the PSA serum test, surgical procedures are performed in the early stage of the disease. The coexistence of primary prostatic malignancy with another neoplasm is rare and usually referred to as a collision of urinary bladder carcinoma and prostatic adenocarcinoma (PA) [2, 3]. PA synchronously occurring with non-Hodgkin lymphoma is extremely rare. To our knowledge, this is one of the newer reports describing PA with incidental finding of mantle cell lymphoma (MCL) in pelvic LNs.

Case report

A 68-year-old male was presented to our tertiary care institution due to an elective radical prostatectomy. The procedure was planned after the patient's core needle biopsy. Anamnestic data showed only a history of urination difficulties and occasional urinary hesitancy. The patient did not state any previous disease or medicine consumption. An initial physical exam showed prostate enlargement with palpable irregularities in shape. Laboratory investigation revealed elevated PSA levels (14 ng/mL; reference range up to 4.5 ng/mL in the age group 60–69 years). Based on the previous examination, a core needle biopsy was performed, and the pathohistological diagnosis of PA was confirmed, with the Gleason score 3 + 4 = 7 and ISUP Grade Group 2.

Due to the anamnestic data, physical exam, PSA level, and pathohistological diagnosis, the patient was planned for radical prostatectomy. During his stay at the hospital, preoperative examinations showed that his heart and pulmonary conditions were within normal limits. Multislice computed tomography (CT) scan of the abdomen and pelvis was performed to visualize disease extension. This imaging method showed a tumor mass confined to the prostate but also revealed excessive LN enlargement.

Intraoperatively, the enlargement of both right and left parailiac LNs, right and left obturator LNs, presacral LNs, and those around common iliac veins was noted. The enlarged LNs were removed, along with the prostate, urethral and urinary bladder margin, as well as the small amount of adjacent adipose and connective tissue.

The excised specimens were sent for histopathological examination and definite diagnosis. On gross examination, surgically marked LNs from different pelvic lodges were described as fragments of fibroadipose tissue. Serial sectioning showed a homogenous appearance of many nodes that were white to greyish, with a soft consistency and in different diameters. The prostate was 6 cm in greatest diameter and grossly described as having a partly homogenous appearance and spongy consistency on serial sectioning. Posterior parts of both lobes were described as ill-defined from the rest of the prostatic tissue, white to yellowish, and with elastic to a firm consistency.

Histopathological (Figure 1) and immunohistochemical (IH) (Figure 2) examinations of LNs and prostatic tissue were performed in order to establish a more precise diagnosis. Histologic material was reviewed by two

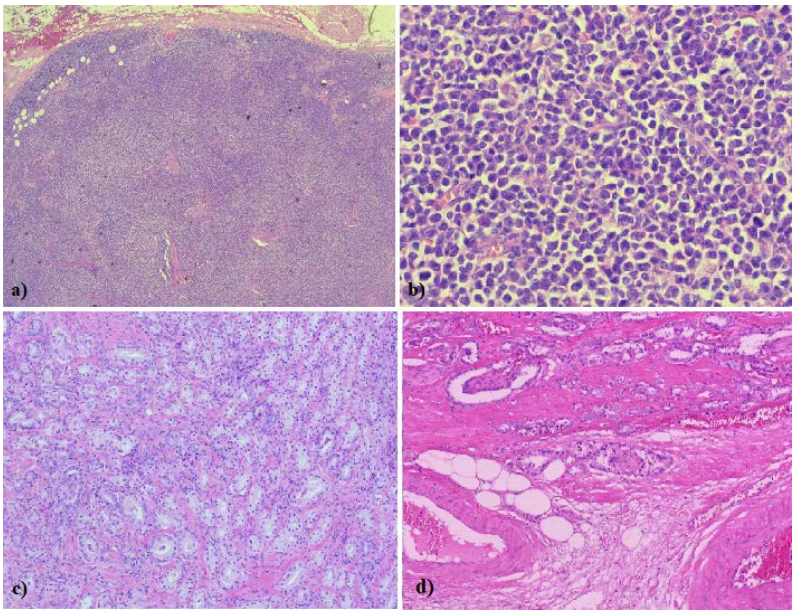

Fig. 1 – a) Mantle cell lymphoma (MCL) in the pelvic lymph node, hematoxylin and eosin (HE) staining, x25; b) Diffuse proliferation of small and irregularly shaped lymphoid cells in MCL, HE, x40; c) Prostatic adenocarcinoma, Gleason score 3 + 4 = 7, HE, x10; d) Extraprostatic extension of adenocarcinoma, HE, x40.

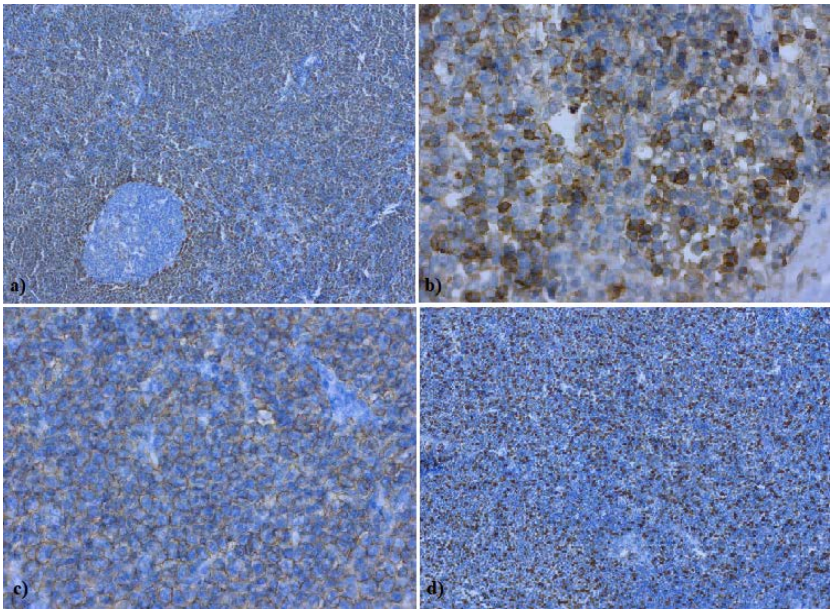

Fig. 2 – Immunohistochemical analysis of selected lymph node specimens showed: a) Cyclin D1 positivity, x10; b) CD5 positivity, x40; c) CD20 positivity, x40; d) Ki-67 proliferative index, x10.

pathologists. Microscopic examination of 25 tissue specimens in 50 histological sections, primarily stained with hematoxylin and eosin (HE), showed 32 LNs with no metastatic deposits originating from PA. However, the LNs showed no recognizable architecture due to the neoplastic tissue with a nodular growth pattern (Figure 1a). The tumor tissue showed diffuse proliferation of small and irregularly shaped lymphoid cells with irregular nuclear borders, clumped chromatin, and inconspicuous nuclei (Figure 1b). The mantle zone was extended. Rare epitheloid histiocytes were present. Stromal vessels were hyalinized. IH evaluation was performed in order to confirm the diagnosis. The tissue specimen selected for IH staining was CD20 positive, BCL2 positive, CD5 positive, CD43 positive, cyclin D1 positive, CD3 negative, CD10 negative, and BCL6 negative. Ki67 proliferative index was up to 20% (Figure 2a–d). Based on the clear morphologic appearance and IH staining, the diagnosis of MCL – the classic variant, was given.

Microscopic examination of prostatic tissue specimens showed multiple focuses on tumor tissue. The tumor tissue consisted of small, uniform, and well-formed glands, as well as focuses with distinct infiltrative growth patterns and glands that were ill-defined or fused, with little or no intervening stroma, and in cribriform or glomeruloid appearance. Tumor cells were atypical, mostly cuboidal, with hyperchromatic nuclei and prominent nucleoli. Based on the microscopic appearance and its correlation with gross description, a diagnosis of acinar adenocarcinoma was made. Histologic grade was defined as Grade Group 2 (Gleason score 3 + 4 = 7). Tumor quantitation was defined by percentage, with 21–30% of the prostate being involved by tumor and with Grade 4 present in 31–40% of the tumor tissue (Figure 1c). Tumor tissue was found in specimens from the right and left lobe, left base, and right apex. In a few focuses of the right lobe sections, small tumor glands were noted beyond the confines of the prostate gland, so extraprostatic extension was defined as focal (Figure 1d). Urinary bladder neck and seminal vesicle invasion were not identified, nor was the lymphovascular invasion. Perineural invasion was identified in specimens from the right lobe and right apex. The surgical margin involved by invasive carcinoma was right apical, Gleason pattern 3. As all regional LN s were negative for acinar adenocarcinoma, the pathologic stage was defined as pT3aN0 [4].

During his 7-day stay at the hospital, the patient was asymptomatic, and there were no abnormalities in clinical examination or basic laboratory parameters.

After a short period of time, the patient had multiple control examinations where he presented in stable cardiopulmonary condition, with no signs of the anemic or hemorrhagic syndrome but with palpable neck, axillar and inguinal LNs, and splenomegaly during his last examination at the Clinic for Hematology. In order to evaluate extranodal involvement with the disease, a bone marrow biopsy was scheduled. Microscopic analysis of the biopsy confirmed bone marrow infiltration with MCL and confirmed the diagnosis given after LN dissection during radical prostatectomy. Stage IV of lymphoproliferative disease was given. The chest, abdominal and pelvic CT showed generalized lymphadenomegaly with the predominance of retroperitoneal LNs swelling and splenomegaly. With these results, the patient was presented to the council for therapeutic management of lymphoproliferative diseases, where it was decided that the patient would be treated by following the Knospe treatment protocol, with an adequate dosage of chlorambucil (tablet 2 mg, 6 + 5 + 5, 1st and 15th day of the cycle) and prednisone (tablet 20 mg, 1 + 1+ 1/2). Since the patient was not motivated by the available treatment options, he did not come to the next scheduled follow-up examination, so further clinical data are unavailable, unfortunately.

Discussion

In the present study, we describe an unusual case of two neoplasms simultaneously occurring in a 68-year-old patient. HE and IH staining of tissue sections, with a demonstration of markers for the targeted lymphoproliferative disorder, supports the diagnosis. In our case, there were no previous signs of an underlying lymphoproliferative disease. Using an imaging method (CT) as a part of preoperative preparation, the patient was diagnosed with a pelvic lymphadenomegaly, not otherwise specified.

MCL is a B cell neoplasm arising from pregerminal center cells of primary follicles or from the mantle zone of secondary follicles. The median age of MCL presentation is 60 years, with a range from 35 to 85 years, as it is approximately in PA [5–7]. The most common involvement site is a LN, with no tendency to infiltrate a particular group of LNs [7]. Morphologically, MCL is present through the expansion of the mantle zone, which surrounds the germinal center. The monomorphic lymphocytes are small to medium-sized, with scanty cytoplasm, irregular nuclear contours, dispersed chromatin, and often inconspicuous nucleolus [8]. They express a variety of B cell antigens, such as CD5, CD19, CD20, CD22, and CD45. The immunopositivity of cyclin D1 is pathognomonic if present [6–8].

The cytogenetic abnormality marking MCL is (11; 14) (q13; q32) translocation between the immunoglobulin heavy-chain locus (IGH) gene on chromosome 14 and the BCL1 locus on chromosome 11. This gene rearrangement results in CCND1 overexpression and potentially increased half-life of cyclin D1. These changes lead to a loss of cell cycle regulating elements, i.e., Rb1 and p27, resulting in the development of MCL [5, 7, 9].

In the case of PA, most patients are asymptomatic. They rarely exhibit symptoms and signs related to the metastatic disease, most commonly in regional LNs and bones [1]. The disease can be managed in a number of ways, such as active surveillance, radical prostatectomy, hormonal therapy, radiation therapy, and cryotherapy. The performance status of a patient should be evaluated, and the morbidity related to surgery and/or chemoradiotherapy should be estimated [1, 7]. The prognosis is expected to be favorable for prostate cancer that is well to moderately differentiated and confined to the prostate gland, and a 5-year outcome is considered excellent [1].

The clinical impact of hematolymphoid malignancies discovered after radical prostatectomy and regional LN dissection has not been completely clarified due to rare cases presented in the literature. Along with individual case reports, there are larger surgical series describing the frequency of the simultaneous presence of PA and lymphoma or leukemia and the possibility of further treatment. In the study by Terris et al. [10], 1,092 patients underwent radical prostatectomy and LN dissection, but only 13 (1.2%) of them had hematolymphoid malignancies, including Hodgkin's lymphoma, hairy cell leukemia, and chronic lymphocytic leukemia. Nine out of thirteen patients had prostatic and LN involvement with lymphoma/leukemia, and LNs were involved in only four patients. An interesting fact is that patients with prostatic involvement with hematolymphoid malignancy had no ultrasound-detected abnormalities other than those corresponding to PA. Similar results were presented in the study by Chu et al. [11], where authors evaluated 4,381 prostatic tissue specimens obtained by biopsy, transurethral resection, or prostatectomy. Only 29 (0.6%) lymphoma cases involving prostate and pelvic LNs were identified, but 11 patients had concurrent known lymphoma. Petković et al. [12] presented a case report where MCL was first discovered as bone marrow infiltration and with the IVB stage of the disease. The coincidental finding of these malignancies led to the patient being double-treated by two separate methods – the CHOP (cyclophosphamide, hydroxydaunorubicin, Oncovin, prednisone) chemotherapeutic protocol and hormonal therapy.

The coexistence of adenocarcinoma and MCL in other anatomical sites, such as the lung, has been reported in the literature. Braham et al. [13] presented the first case of primary lung adenocarcinoma associated with LN MCL. This lymphoma is usually synchronously present with plasma cell dyscrasia or granulomatous diseases such as sarcoidosis [14, 15]. It can also occur with metastasis from a different anatomical site but in the same LN [9]. In addition, there were reports describing the presence of two different variants of MCL in the same LN, usually classic type and blastoid variant [8, 9]. MCL has a wide differential diagnosis, mostly including other lymphoproliferative disorders that can mimic the presence of MCL, especially reactive follicular hyperplasia. Differential diagnosis also includes chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, nodal marginal zone B cell lymphoma, splenic marginal zone lymphoma, as well as Castleman disease [16, 17]. There were several studies describing concomitant PA and chronic lymphocytic leukemia, follicular lymphoma, and B cell lymphoma. The results of these studies point to different therapeutic management due to the various age range, patient's general status, and prognostic parameters, with the stage being the most important one. Furthermore, the results from follow-ups showed different prognoses for these patients. Most frequently, patients presented with recurrent prostatic carcinoma [12, 18–21].

Conclusion

Synchronous development of these tumors can be a challenging problem in diagnosis and treatment. The therapeutic management required separate consideration due to their different biological behavior. In the case of the presented patient, pelvic LN enlargement discovered on a CT scan was assessed in order to determine if the pelvic lymphadenopathy corresponded to metastasis or a separate process in the LN. In the absence of metastatic adenocarcinoma, the prostatic tumor was treated first due to its stage, and then MCL was treated. Even though there is no metastatic disease, careful examination of the regional LNs is imperative for a proper diagnosis and staging.

Conflict of interest

The authors declare no conflict of interest.

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General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ‖, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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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Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper left corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

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Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

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Abbreviations and acronyms

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An alphabetical list of all abbreviations used in the paper, followed by their full definitions, should be provided on submission.

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U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

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Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

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b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

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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.

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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.

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

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Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

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

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

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.

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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.

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