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THE FIRST ISSUE WAS PUBLISHED IN 1948

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Med Pregl 2017; LXX (11-12): 339-474. Novi Sad: November-December.

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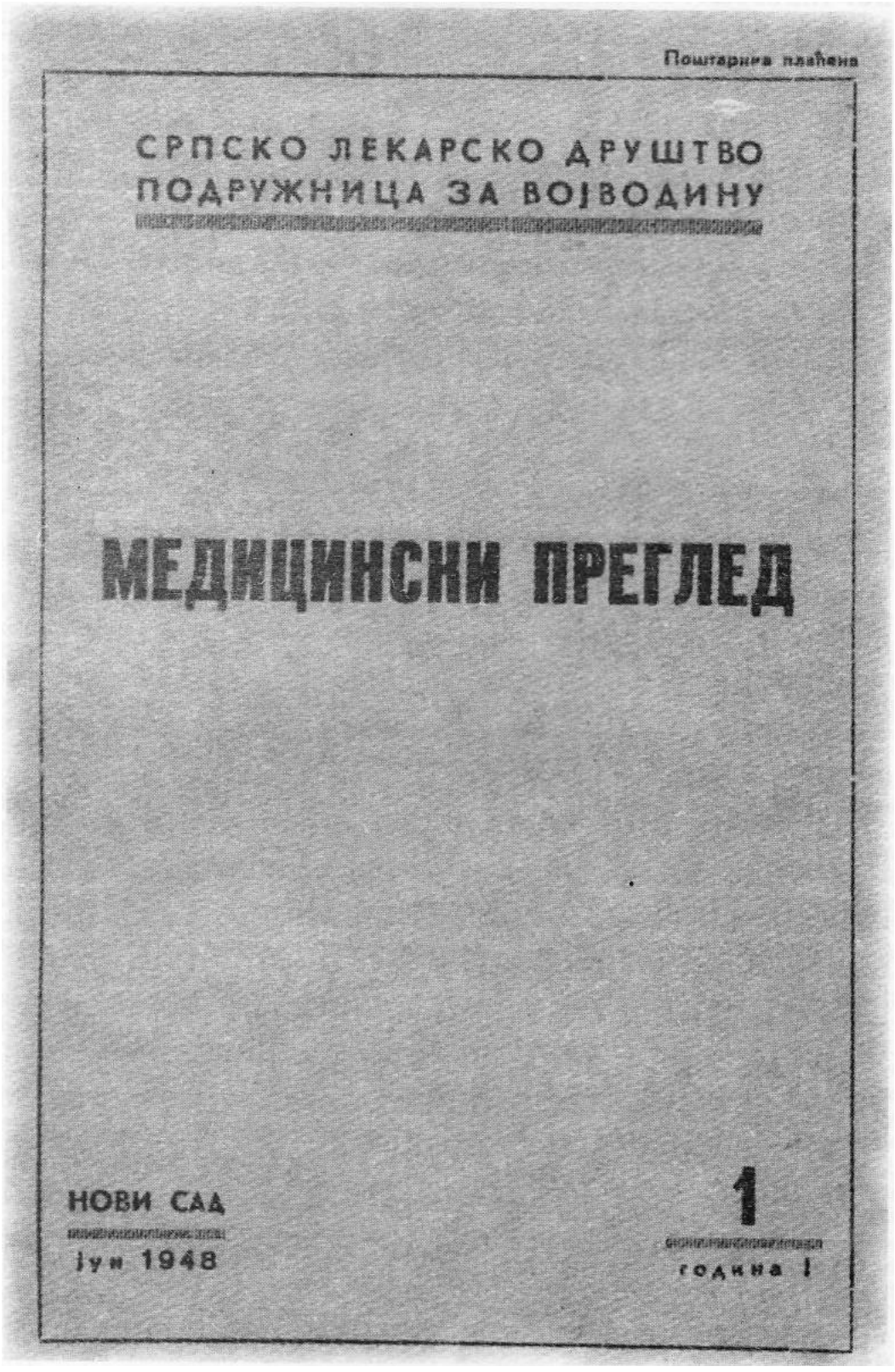

Front page of the first issue of the journal "Medical Review"
Naslovna strana prvog broja časopisa "Medicinski pregled"

EDITORIAL
UVODNIK

University of Novi Sad, Faculty of Medicine Novi Sad, Department of Anatomy

Uvodnik
Editorial
UDK 070.486:61"1948/2018"

THE SEVENTIETH ANNIVERSARY OF PUBLICATION OF THE *MEDICAL REVIEW*, 1948 - 2018

SEDAMDESETGODIŠNJICA IZLAŽENJA ČASOPISA MEDICINSKI PREGLED, 1948-2018

Ljilja MIJATOV UKROPINA

In 2018, the *Medical Review* celebrates 70 years of continuous publication. It is certainly an important jubilee, a significant mark and a lasting reminder of the time that has passed.

In July 1948, the first issue of our journal was published, as a result of the initiative of the Main Provincial Hospital's physicians, members of the *Society of Physicians of Vojvodina* in Novi Sad. The crucial year for the journal was 1953: it started being issued on regular basis, not occasionally, and its current appearance dates back to the same year. This tradition is still respected, including almost all the sections as seven decades ago, with the introduction of abstracts in the world languages, thus being recognized in a wider area. It remains to be noted that in the bold visionary venture that enabled the publication of our journal, the greatest contribution was given by Dr. Vladimir Jakovljević, the first president of the Editorial Board, Prof. Dimitrije Stanulović, the first secretary of the Editorial Board, and first Editor-in-Chief, Prim. Dr. Miloš Ćirić. Their efforts were sustained and improved over the years, thanks to a significant number of the most respected colleagues, enthusiasts from our community who showed ambition and ability to support the general interest in medical science and health care.

As a medical journal, *Medical Review* plays an important role in the creation of medical science and medical practice. It is intended for a wide range of physicians from various fields of biomedicine as a source of new knowledge used in the treatment of patients. In spite of numerous difficulties, primarily financial, the *Medical Review* has been constantly improved and updated. The papers published in it are indexed in several secondary publications, of which *Medline* is the most important. In addition to the leading domestic health professionals, the journal's editorial board also includes renowned experts from abroad. The number of papers submitted for publication has increased from year to year. The National Library of Serbia has enabled the allocation of the DOI (Digital Object Identifier) numbers to each paper, thereby increasing its visibility and providing permanent access to full articles published in the journal on the Internet. During 2008 and 2009, the *Medical Review* was classified as a journal of international importance - M24, according to the categorization of medical journals carried out by the Medical Science Committee of the Ministry of Science and Technology. During this period, the number of submitted papers was so great that the number of published articles was doubled, in order to avoid longer waiting periods and ensure timely publication of the achieved results.

However, in 2009, the journal was moved to the category M51, in our opinion without clear and insufficiently justified reasons. After these changes, the number of submitted papers has decreased, due to the well known conditions for the election to teaching and scientific titles. Nevertheless, the members of the editorial board continued working with the same energy in order to maintain the continuity of the journal. For this reason, since 2013, the papers are published in English, and abstracts both in English and in Serbian. The editors signed a contract with the *Center for Evaluation in Education and Science* on the use of the ASEESTANT for submission and review of papers online. The main goal of using this service is to improve the editorial efficiency, the quality and regularity of the peer review procedure, for preventing duplicate publication and plagiarism, improve the equipment and, based on all this, improve the quality and impact of the journal. Authors and reviewers are giving their great contribution to our journal, and we owe a great

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deal of appreciation for their recommendations and remarks.

The 70th anniversary of *Medical Review* is definitely an enviable success and a reason for celebration, but in the light of a realistic view of the present, it is still necessary to invest efforts of all who are gathered around the publishing activity of the *Society of Physicians of Vojvodina* in improving the quality of published articles and journal promotion.

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CONTEMPORARY ANATOMY TEACHING – EXPERIENCES FROM THE FACULTY OF MEDICINE NOVI SAD

SAVREMENA NASTAVA ANATOMIJE – ISKUSTVA MEDICINSKOG FAKULTETA NOVI SAD

Biljana SRDIĆ GALIĆ, Mirjana UDICKI, Nikola VUČINIĆ, Dragana RADOŠEVIĆ, Sonja ŽIGIĆ, Zorka DRVENDŽIJA, Radmila PERIĆ, Bojana KRSTONOŠIĆ, Ljilja MIJATOV UKROPINA, Ljubica STOJŠIĆ DŽUNJA, Dušica MARIĆ, Mirela ERIĆ and Siniša S. BABOVIĆ

Summary
The expansion of medical knowledge supported by rapid progress of information technologies led to important changes of medical education concept, as well as volume and content of medical curriculum. Anatomy teaching has gone through many restrictive measures. First, the number of lessons has been reduced and subsequently focus from systematic and regional anatomy has been moved to clinical anatomy with the aim of practical integration of knowledge in clinical conditions. Today the justification of cadaver dissection that is considered to be the backbone of traditional anatomy teaching is being questioned. Numerous problems are associated with the cadavers use in teaching, including their accessibility, preservation and ethical justification. Cadaver dissection has alternatives in real anatomical models, as well as in virtual multimedia, ranging from simple linear to sophisticated, interactive, augmentative reality. Although, the use of modern technologies is estimated by some lecturers as the act of dehumanization of medicine and criticized as insufficiently student oriented, it is considered justified only as a supplement to traditional dissection method and demonstration on cadaver material. Anatomists are put in front of very delicate task to integrate the positive aspects of traditional and modern teaching, with the aim to motivate students and provide conditions for better knowledge retention. This paper deals with problems and challenges facing contemporary anatomy teaching from the perspective of the Department of Anatomy, Faculty of Medicine Novi Sad.
Key words: Education, Medical, Undergraduate; Anatomy; Cadaver; Dissection; Ethics; Educational Technology; Curriculum

Sažetak
Ekspanzija medicinskog znanja, podržana rapidnim napretkom informaciono-komunikacionih tehnologija dovela je do bitnih izmena koncepta medicinske edukacije i obima i sadržine medicinskog kurikuluma. Nastava predmeta Anatomija podlegla je brojnim restriktivnim merama. U prvom redu došlo je do redukcije broja časova, a potom i do pomeranja težišta sa sistematske i regionalne na kliničku anatomiju, sa ciljem praktične integracije znanja u kliničkim uslovima i razvijanja veštine rešavanja kliničkih problema. Danas se preispituje i opravdanost disekcija kadavera koja predstavlja okosnicu tradicionalne nastave anatomije. Brojni su problemi povezani sa korišćenjem kadavera u nastavi – od njihove dostupnosti, prezervacije, pa do etičke opravdanosti. Disekcija kadavera dobila je alternative u realnim anatomskim modelima, kao i u virtuelnim, multimedijalnim sadržajima – od jednostavnih, linearnih pa do sofisticiranih tehnologija interaktivne, „proširene“, realnosti. Ipak, primenu modernih tehnologija neki ocenjuju kao akt dehumanizacije medicine i kritikuju je kao nedovoljno orijentisanu ka studentu, smatrajući da je opravdana samo kao dopuna klasičnim metodama disekcije i demonstracije na kadaveričnom materijalu. Pred anatomima je delikatan zadatak da integrišu pozitivne aspekte tradicionalne nastave sa modernom, sa ciljem motivacije studenata i obezbeđivanja uslova za bolju retenciju znanja. Ovaj rad se bavi problemima i izazovima sa kojima se sreću predavači savremene nastave predmeta Anatomija iz ugla Zavoda za anatomiju Medicinskog fakulteta Novi Sad.
Ključne reči: osnovne studije medicine; anatomija; leš; disekcija; etika; obrazovna tehnologija; kurikulum

Introduction

Over the past decades rapid technological development has resulted in inevitable reforms of the entire school system, including higher education as well. Modern medical curriculum is more problem-oriented, integrated and based on the use of modern technologies. The traditional border between preclinical and clinical disciplines has disappeared, and conditions for their interrelations have been created. In the light of the above mentioned changes, the teaching of anatomy has undergone significant changes of scope, content and teaching methods. Traditional dissection and demonstration on cadaveric material gave way to modern imaging methods and virtual reality, while the content has been largely clinically oriented. These changes at medical schools in Serbia coincided with the beginning of the application of the principles of the Bologna Declaration. Introducing new study programs at the Faculty of Medicine in Novi Sad after the year 2000 and higher education reforms represented big challenges to the Department of Anatomy. The aim of this review is to assess the position of anatomy in

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the modern medical curriculum, considering experience from the Department of Anatomy of the Faculty of Medicine in Novi Sad.

Traditional vs. Modern Medical Curriculum

Traditional medical curriculum is largely criticized because of the lack of interactivity and learning out of context. It is based on the classical teaching forms where the teacher is in the center and students are passive recipients [1–3]. Modern medical curriculum has been adjusted to the needs of the students of a new millennium. "Millennials" is a term widely used for generations born from the 1980s to the present, grown in conditions where digital technologies are part of their everyday lifetime. Based on these data, as well as the situation in society and education, it can be concluded that electronic media dominate their lives for many reasons, first of all because they provide visualization, dynamic forms and nonlinear sequences of knowledge, active and interactive communication, and most importantly, the fear of pedagogical sanctions that are so typical for a traditional school. Students of the new millennium are distinguished by impatience, requisite for quick availability of information sources, short-term engagement with a topic, a quick shift from theme to topic, sharing attention - doing several things at the same time (learning from other information), priority - unprinted digital resources (images, movement, music, not text), new language - abbreviations, exposure to a very well-designed and exciting context. This is the main reason of the "generation gap" between students and teachers nowadays. Prensky called the students "digital natives" and teachers "digital immigrants" [4]. Therefore, different learning models have been designed to respond to the abilities of the millennials. Motivation has a key impact on the effectiveness of learning for the students of the new millennium [5]. Modern medical curriculum puts the student in the centre of the learning process; it becomes more problem-based which stimulates students to be more active, and makes learning process more relevant. It is also integrated which means that the content of the basic, preclinical subjects is placed in the context of the clinical practice [1–3, 6]. Those changes of the medical curriculum largely influenced on the content of the anatomy. Due to worldwide undergraduate reforms, many programs have decreased the time spent on basic sciences, and anatomy has suffered greatly.

Anatomy Teaching – then and now

Anatomy training has always served as an essential part of any medical curriculum, regardless of the institution [7]. In the earliest medical programs on record, anatomy itself was enough to constitute a complete preclinical education. Though it's now part of a more comprehensive medical training approach, anatomy still serves as the conceptual framework for general medicine as well as specialties like surgery and radiology [8]. Famed Scottish surgeon Robert Liston was once quoted as saying "The art of operating must be laid in the dissecting room" [9].

Over time, anatomy's status as a science has changed. Early anatomists founded the study of anatomy using a combination of data collection (i.e. directly observing body structures), hypothetical explanation, and observational hypothesis testing. But today, it's highly unlikely that new gross structures will be discovered, except for the neuroanatomy field. The lack of possibility of discovery has made anatomy more of an objective reality [10]. The anatomical body outlined in medical textbooks is presented as a grouping of common observations, often in a literary structure that's both easily understood and so complex it could be studied for a lifetime. Learning, but not discovering, anatomy is a reality that, when studied and later appreciated by everyone, becomes part of a common experience [11]. Traditionally, anatomy has been taught descriptively and topographically, based mainly on the cadaveric dissections. Nowadays, it is unfairly proclaimed as archaic, traditional, passive and too factual subject. All over the world anatomy content was reduced and cadaveric dissection has been largely replaced by virtual reality, while the content became more functional and clinically oriented. Three fundamental questions regarding to current anatomy teaching are addressed: how much, when and how anatomy should be learned [12].

Minimum anatomy knowledge should be enough for an independent practitioner to practice safely. Older et al. stated that there is sevenfold increase in claims associated with anatomical errors submitted to the Medical Defence between 1995 and 2000 [13]. Several studies showed poor anatomical knowledge among students, young doctors [14–16] and clinicians [17, 18]. Facts like this alerts anatomists to raise the question if the anatomy knowledge has fallen beneath safe level [13].

Traditionally, anatomy has been taught in the first undergraduate year. Because of that, future doctors and specialist have only one opportunity for anatomy learning, and that is not enough. Retention of basic science knowledge has to be discussed in the light of a "negatively accelerated (logarithmic) forgetting curve" [19]. Approximately 25% of knowledge is lost after one year [20]. A recent report of 5th year chiropractic students in Australia reported that only 38% of the cohort was able to accurately identify all eight carpal bones [21]. Solution would be to integrate anatomy during the whole studies and postgraduate and to provide optimal number of anatomy hours.

At the beginning of the 20th century students attended about 500 hours of anatomy, at the half of the last century about 300, and in the end, beneath 200 hours [22]. Course hours, not including examination ones, at the Faculty of Medicine Novi Sad

in the school year 2005/06 amounted 150 lecture hours and 165 hours of practice. Anatomy took three semesters and it was divided into two subjects: Anatomy 1, which was held during the first year (amounted 135 lecture hours and 150 hours of practices), and Anatomy 2, that included anatomy of the central nervous system and special senses, that was taught in the second year of medical studies (amounted 15 lecture hours and 15 practical lessons). Today, there is only one subject that takes two semesters; the number of hours fell on 135 lecture hours and 150 practical hours (285 in total) and it awards 24 ECTS. Study material is shortened and partially adjusted to the clinical needs but students lack the proper neuroanatomical knowledge because of the insufficient time to master such a complex discipline. Similar situation is at the other medical faculties in Serbia: number of total anatomy hours is between 255 and 280, and they weight between 16 and 24 ECTS.

According to the European Federation for Experimental Morphology (EFEM) – Consensus Conference on Morphological Sciences 2007 the total weight of morphological sciences (anatomy, histology and embryology) should not fall below the 30 ECTS [23]. The same resolution recommends vertical or horizontal integration of morphological subjects, or teaching morphology as an introduction to clinical matters rather than basic education in morphology during the first years of the curriculum. Moore et al. also recommended anatomy not to be completed in the first years of medical school, but to continue in the following years by means of optional and facultative subjects such as clinical anatomy, imagining anatomy, or joint biomechanics [24]. This model is partially applied at the Serbian universities including Faculty of Medicine Novi Sad where students of the second and third year are offered two anatomy electives: Variations in Anatomy and Clinical surface anatomy; these subjects provide clinical insight into the anatomy knowledge enabling the continuity between preclinical and clinical subjects. Given the existence of a large number of variations of organs, muscles, their vascularization and innervation, knowledge about them is essential for physicians in practice, which justifies the introduction of this course to basic medical studies. Gaining knowledge about the surface morphology of the body and the projections of the internal organs and structures on the body surface will form the basis for clinical propedeutics and radiology as well as the possibility of applying the acquired knowledge of the observed objects in all morphological branches of medicine.

Teaching Methods in Anatomy

One of the greatest sources of modern debate in medical education concerns using computer tools as part of the curriculum [25]. All over the world lectures based on the use of chalk and board are still the most represented ways of teaching although lately many forms of computer assisted techniques are present. Anatomy represents the area in which computer tools have made a marked difference.

The use of computer assisted techniques is available with the help of computer science development and is mostly accepted by younger teachers and researchers who are familiar with them. Among these techniques by far the most consumed are Power Point presentations. Both traditional lectures with chalk and board as well as the modern ones supported by Power Point presentations have their own advantages and disadvantages.Though several studies have attempted to uncover the benefits of computer-assisted learning, there's still little proof that it has a stronger impact than traditional teaching methods – traditional lectures appeared to provide better interaction between students and teachers [26-33]. To date, most technological changes introduced have been complements to traditional teaching methods instead of replacements. Computer tools have been used as sources of consultation, study materials for medical students, and even as support materials for attendance-based teaching [34, 35].

Since 2000, the Medical Faculty of the University of Novi Sad has integrated computer-based materials (i.e. lesson summaries with hyperlinked images, test examples, lesson key points and instructions, etc.) into its more traditional attendance-based anatomy courses. The university disseminates these materials via virtual campus, which mainly serves as a complement to course lectures and as a follow-up for lessons or activities covered in the courses. Some of the materials have been designed as teaching aids (i.e. Power Point presentations, animations, videos, etc.) to help professors deliver more effective anatomy lectures.

Practice in Anatomical Education – from Cadaver Dissection to Virtual Reality

Traditional cadaver dissection had inception in ancient Greece in 3rd century BC. Through the centuries it has been recognized as important method in anatomy studying, giving students a 3D view of human anatomy and developing a self-directed learning and team framework [36]. In the 21st century there is a great tendency to use the computer technologies and new pedagogical methods instead of performing human dissection as the overpassed, old-fashioned anatomical teaching tool. The positive sides of traditional cadaver dissection could deny this tendency, marking the human dissection as necessary practice and core part of anatomy education. Human dissection gives students the opportunity to learn independently about human body structures and their relationship, acquisitioning the manual skills and getting insight into anatomical variations. Students question they view of death and dying, confronting maybe for the first time the real human mortality in front of human body remains [37], and gaining a

first-hand appreciation of human life [38]. However, the method of traditional cadaver dissection brings a lot of ethical, technical, health and other problems, which have been discussed in entire scientific and laity community. On the other hand, due to the lack of cadavers, the demonstration on cadaveric material has been used at universities worldwide as the equally good method of anatomy learning. For the same reason medical students at the University of Novi Sad do not do the dissections themselves, but they have been taught by the method of demonstration on cadaveric material, or using additional materials like anatomical models and images.

Donating body for research purposes in anatomy is still relatively rare, and many countries have instituted programs and regulations surrounding the donation of cadavers or body parts. Using publication and lecture meetings, people worldwide are getting more informed about body donation, and from the second half of 20th century the raise of body donation programs as the source of human cadavers for anatomical dissection has been recorded [39]. The decision to become a body donor is influenced by factors as social awareness, altruism, desire to aid the advancement of medical knowledge and funeral cost savings [40]. The reasons for unwillingness to donate the body are family concern, not being psychologically ready, anxiety of mistreatment the body and religious beliefs [41]. The lack of human cadavers remains a great issue at medical faculties in some universities worldwide, including the Faculty of Medicine Novi Sad [42].

Another disadvantage of using cadavers is the problem of conservation and storage. Preparing of cadavers for dissection is a really long process, and afterwards the cadavers have to be kept in special conditions, which is expensive and time consuming. Beside those disadvantages of human dissection, there is also one concerning the health - students and teaching stuff are confronted to the health hazard of formalin, which is proved to be acute and chronically toxic [43]. In the year 2012 the Department of Anatomy of the Faculty of Medicine Novi Sad was reconstructed **(Figure 1)**. This project was realized within the Infrastructural Programme for Higher Education proposed by the Ministry of Education and the Ministry of Science and Technological Development within the process of programming the European Union funds (IPA – Instrument for Pre-Accession Assistance) with the following aims: improving the quality of teaching and research-scientific work in the field of anatomy, improving safety and health of students and staff and environmental protection. It comprised reconstruction, rehabilitation and adaptation of facilities for storage and processing of cadaver materials aimed at reducing concentration and emission rate of formalin, installation of modern ventilation system with filters for formalin fumes in teaching rooms and hallway and adapting the facilities and purchasing the equipment for plastination technique **(Figure 2)**. Plastination is a relatively new technique of conserving the cadaveric material, which can be kept in the air. This method was created by Gunther Von Hagens in Heidelberg University in Germany in 1978, and it is currently used worldwide in both teaching and research [44–46]. This method provides conserving cadaveric material by replacing the water and fat in tissues by curable polymers (silicone, epoxy, polyester), resulting in hard, dry and durable specimens that can be touched and even retain most properties of the original sample. The great advantage of this preservation method is absence of proved health hazards, like the ones that the traditional human dissection brings with the use of formalin.

Figure 1. Ventilation system in the dissection hall of the Department of Anatomy of the Faculty of Medicine Novi Sad – before and after reconstruction
Slika 1. *Ventilacioni sistem u salama za disekciju Zavoda za anatomiju Medicinskog fakulteta Novi Sad – pre i posle rekonstrukcije*

The human dissection has some demonstration disadvantage, which reflects in unreal representation due to the loss of properties during the fixation, and in the representation of small structures (e.g. the ear). Also, it is not to neglect the emotional conflicts with body donors and their families.

The ethical question about the necessity of human dissection in medical studies was raised by many anatomists and the laity. The issue is: are medical students respectful enough in front of the human remains, being aware of the fact that all the cadavers

Figure 2. Plastinated specimens made at the Department of Anatomy, Faculty of Medicine Novi Sad
Slika 2. *Plastinirani preparati načinjeni na Zavodu za anatomiju Medicinskog fakulteta Novi Sad*

were persons? In Japan, for example, medical students before the dissection first pray for the souls of the donors, with deep understanding of good will of donors and the feelings of their families [47].

Most published papers on anatomy curriculum strongly recommend incorporating dissection. Dissection provides students an array of benefits, mostly in the domains of skills, attitudes, and knowledge acquisition and integration [11]. The majority of anatomists agree that the dissection has passed the greatest pedagogical test, which is the test of time. The dissection has survived numerous historical turbulences, cultural changes and pedagogical trends. Using this traditional with other technological resources, students should develop skills in three categories: theoretical, practical and bioethical [48].

Many medical schools and anatomy departments have investigated alternatives to cadaver-based instruction like two-dimensional (2D) and three-dimensional (3D) imaging [49] or using 3D anatomical models.

Imaging methods have been used as practical tools of learning to recognize the human anatomical structures and their relationship inside the body. The radiography, computed tomography, magnetic resonance imaging, positron emission tomography and ultrasonography give the opportunity to present anatomical structures in axial, sagittal and coronal planes, with the possibility to remove irrelevant tissues, such as bones. Because images can be recorded and stored digitally, they are adequate for analyses in detail during teaching. The technological innovations brought us a great range of interactive multimedia used as individual anatomy learning methods – the 3D atlases software, the virtual dissection, the interactive 3D anatomy boards, augmentative reality with somatosensory response, etc [49]. A great disadvantage of these technological innovations is the price, which makes them unavailable to many medical faculties worldwide. Biassuto et al. compared the capability of cadaver dissections and computerized resources to provide proper understanding and learning anatomy – their results showed better results in the traditional teaching group than the technologically supported group, evaluated by the number of students that passed their exams, while the group that followed the course with both practical resources was better than the others [50]. Even when computerized improvements have developed a new area giving students a lot of elements to facilitate their approach to imaging structures, it was shown that the possibility of direct contact with cadaveric material cannot yet be replaced. This study has demonstrated that the best possibility for learning anatomy is interaction of all these resources. The use of modern technologies is estimated by some lecturers as the act of dehumanization of medicine and criticized as insufficiently student oriented. It is considered justified only as a supplement to traditional dissection method and demonstration on cadaver material.

Three dimensional printing of prosected specimens' products highly realistic 3D replicas **(Figure 3)**. With this technique, even small vessels and nerves could be readily distinguished. Furthermore, printing of negative space such as air sinuses and coronary vessels segmented from computed tomography data sets was as anatomically accurate as the original clinical radiological data. It is possible to scale up or scale down in size of the 3D prints and to produce highly satisfactory replicas of dissections, and negative space prints [51]. Arguably the most notable advantage regarding printing reproductions of anatomical dissections is reflected in the superiority of the 3D printed copies to the plastic models. Plastic models are in common use at universities and medical schools. They are mass produced copies or molds which often lack important specific details. They are not ideal for teaching detailed anatomy in medical and allied health professional courses, even if they are suitable for some other teaching purposes.

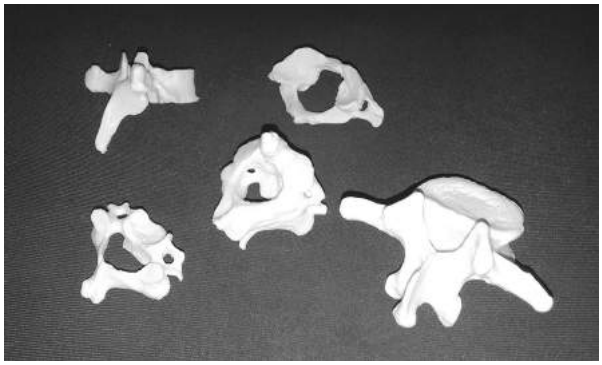

Figure 3. 3D printed vertebrae (from the collection of the Department of Anatomy, Faculty of Medicine Novi Sad)
Slika 3. *3D štampani pršljenovi (iz kolekcije Zavoda za anatomiju Medicinskog fakulteta Novi Sad)*

It is necessary for educators to innovate and change the teaching style and help the students in learning anatomy. There are several creative options for educators, as body painting, modeling clay, wearable art etc. Students can use their anatomy knowledge in modeling or drawing, and also that is the way for better understanding and memorizing complicated anatomical structures and relationships. First modeling course at the Faculty of Medicine Novi Sad entitled "Learning through modeling – Position and relation of mediastinal organs" was organized during the school year 2016/17 **(Figure 4)**. The course was attended by 85 undergraduate students who learned relations between medistinal organs using different materials, such as modeling clay, balloons, wires or crayons. The colorful visual models of various structures that were made by their hands increased their memory. There was a lot of fun during the learning and they were interested in processing other topics, at the same way.

Conclusion

Anatomy teaching at the Faculty of Medicine Novi Sad has been facing many challenges in

achieving the high standards of modern medical curriculum. Transitional period brought many changes and reconsiderations - some of them were hard to bear, but some were creative and refreshing. Concerning positive and negative aspects of traditional and modern curriculum and position of the anatomy in the modern curriculum we feel that it is necessary to retain positive aspects of traditional teaching such is anatomy dissection. On the other hand, clinical orientation makes anatomical knowledge more relevant and helps memorizing. According to the results of several studies modern technologies should be rationally included in the anatomy teaching combining with traditional, formal methods.

Figure 4. Modeling course at the Department of Anatomy, Faculty of Medicine Novi Sad
Slika 4. *Kurs modelovanja na Zavodu za anatomiju Medicinskog fakulteta Novi Sad*

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МЕДИЦИНСКИ ПРЕГЛЕД НОВИ САД, јуни 1948
Година I — Свеска 1

ПОВРЕМЕНО ИЗДАЊЕ СРПСКОГ ЛЕКАРСКОГ ДРУШТВА
— ПОДРУЖНИЦЕ ЗА ВОЈВОДИНУ

ПРЕДГОВОР

Појава „Медицинског прегледа" претставља веома значајан догађај у медицинском животу наше Покрајине Војводине. Замисао о покретању овога повременог стручног медицинског часописа потиче од лекара Главне покрајинске болнице — наше највише медицинске установе у Војводини, а прихватила ју је и остварила Подружница Српског лекарског друштва у Новом Саду.

И поред читавог низа медицинских часописа покренутих у нашој земљи, појава овога часописа није сувишна за нашу медицинску јавност, с обзиром да ће „Медицински преглед", поред основних медицинских проблема, расправљати и специфичне медицинске проблеме у вези са народном патологијом наше Покрајине, а у циљу отклањања штетних уплива по здравље радних маса и подизања здравствене заштите народа на један виши ниво, у складу са општим подизањем животног стандарда у нашој земљи.

Данас, када се брижи о народном здрављу поклања више пажње но икада, „Медицински преглед" преузима на себе корисну улогу, вршећи васпитно-медицински утицај на лекаре Војводине и других крајева наше земље, упознавајући их са новим искуствима и тековинама медицинске науке, која се у нашој Покрајини Војводини, захваљујући нашим болничким лекарима, нарочито високо цени и успешно негује.

Овај часопис омогућиће међусобно упознавање и зближење наших лекара са лекарима других крајева. Они ће на овај начин измењивати мисли и међусобно саопштавати стечена искуства. Наши медицински радници, сарађујући у овом часопису, имаће могућност да своја дугогодишња искуства из медицинске праксе пренесу на млађе лекаре, којима се на овај начин пружа могућност њиховог стручног уздизања.

Повереништво за народно здравље ГИОНСАПВ-а поздравља акцију Српског лекарског друштва на покретању „Медицинског прегледа" који ће, без сумње, успешно остварити замисао наших лекара, да путем свога узвишеног медицинског позива помогну нашем радном народу, чувајући му здравље, пруживши тиме свој снажан допринос у изградњи социјализма у нашој земљи.

Д-р ИВАН БАБОСЕЛАЦ,
Повереник за народно здравље ГИОНСАПВ-а

Preface to the first issue of the journal "Medical Review"
Predgovor iz prvog broja časopisa "Medicinski pregled"

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INCIDENCE OF DIABETIC EYE DISEASE IN ACCORDANCE WITH DURATION, GLYCEMIC CONTROL, BLOOD AND OCULAR PRESSURE

INCIDENCIJA DIJABETESNE BOLESTI OKA U ODNOSU NA TRAJANJE BOLESTI, KONTROLU GLIKEMIJE, KRVNI I OČNI PRITISAK

Vladimir ČANADANOVIĆ[1, 2], Sandra JOVANOVIĆ[1, 2], Sofija DAVIDOVIĆ[1, 2], Ana OROS[1, 2], Vladislav DŽINIĆ[1, 2] and Sava BARIŠIĆ[1]

Summary

Introduction. Diabetic retinopathy remains the leading cause of visual disability and blindness among professionally active adults in economically developed societies, which is of particular concern because the prevalence and incidence of diabetes mellitus is expected to increase sharply during the next decade. There are several known factors responsible for the development of diabetic retinopathy, duration of disease and blood sugar level being the most important ones. **Material and Methods**. Prospective study of 280 diabetic patients (diabetes mellitus type 2) divided into 3 groups according to the duration of diabetes mellitus. All diabetic patients underwent complete ophthalmological examination in artificial mydriasis and optic coherence tomography. A full medical history included patient age, the time elapsed from diabetes diagnosis, current treatment of diabetes, presence of hypertension and glycemic control assessed by glycosylated hemoglobin measurement. **Results.** The mean age of patients was 63.5 years (SD±6.5, range 57-70 years). Mean duration of diabetes was 7.3 years in group I, 12.4 years in group II and 17.2 years in group III. The average value of glycosylated hemoglobin was 6.58% in the group I, 7.64% in the group II and 8.29% in the third group of patients. No statistically significant difference in intraocular pressure and the level of blood pressure were found among groups. Cataract was present in 104 patients (37.1%). Complications related to diabetes among all patients included in our study were: nonproliferative diabetic retinopathy in 48.5%, proliferative diabetic retinopathy in 25.7% and diabetic macular edema in 22.5% of patients. **Conclusion.** The duration of diabetes is one of the most significant factors for the development of diabetic maculopathy and the progression from nonproliferative to its proliferative stage. There is significantly higher incidence of proliferative diabetic retinopathy and diabetic macular edema in patients with increased serum level of glycosylated hemoglobin. Diabetes accompanied by hypertension is related to worsening of the clinical course of diabetic eye diseases and developing diabetic macular edema and proliferative diabetic retinopathy.

Key words: Diabetic Retinopathy; Macular Edema; Cataract; Diabetes Complications; Diabetes Mellitus, Type 2; Blood Glucose; Intraocular Pressure; Hemoglobin A, Glycosylated; Hypertension

Sažetak

Uvod. Dijabetesna retinopatija je i dalje vodeći uzrok pada vidne oštrine i slepila među radno aktivnom populacijom u ekonomski razvijenim društvima, što je od posebnog značaja ako se uzme u obzir da se očekuje povećan broj obolelih od šećerne bolesti u sledećoj deceniji. Poznato je nekoliko faktora odgovornih za razvoj dijabetesne retiniopatije, od kojih su najvažniji dužina trajanja bolesti i visina glikemije. **Materijal i metode.** Ova studija je bila prospektivnog tipa sa 280 pacijenata koji boluju od šećerne bolesti (tip 2), podeljenih u tri grupe u zavisnosti od dužine trajanja bolesti. Svi pacijenti koji boluju od dijabetesa podvrgnuti su kompletnom oftalmološkom pregledu u artificijelnoj midrijazi i snimanju optičkom koherentnom tomografijom. Evidentirana je celokupna istorija bolesti koja je uključivala starost pacijenta, vreme proteklo od postavljanja dijagnoze šećerne bolesti, trenutnu terapiju, prisustvo arterijske hipertenzije, kao i stepen kontrole glikemije procenjena merenjem vrednosti glikoliziranog hemoglobina. **Rezultati.** Prosečna starost pacijenata iznosila je 63,5 godina (SD ± 6,5). Prosečna dužina trajanja šećerne boelsti iznosila je 7,3 godine u prvoj grupi, 12,4 godine u drugoj grupi i 17,2 godine u trećoj grupi. Prosečna vrednost glikoliziranog hemoglobina bila je 6,58% u prvoj grupi, 7,76% u drugoj grupi, dok je ona iznosila 8,29% u trećoj grupi pacijenata. Nije ustanovljena statistički značajna razlika između ispitivanih grupa u visini intraokularnog pritiska i visini krvnog pritiska. Katarakta je bila prisutna kod 104 pacijenta (37,1%). Učestalost komplikacija u vezi sa dijabetesom među ispitivanim pacijentima iznosila je: neproliferativna dijabetesna retinopatija kod 48,5%, proliferativna dijabetesna retinopatija kod 25,7%, a dijabetesni makularni edem kod 22,5% pacijenata. **Zaključak.** Dužina trajanja šećerne bolesti je jedan od najvažnijih faktora rizika za razvoj dijabetesne makulopatije i progresije neproliferativne u proliferativnu formu dijabetičke retinopatije. Ustanovljena je statistički značajno veća incidencija proliferativne dijabetesne retinopatije i dijabetesnog makularnog edema kod pacijenata sa povišenom koncentracijom glikoliziranog hemoglobina u serumu. Dijabetes udružen sa arterijskom hipertenzijom povezan je sa pogoršanjem kliničkog toka bolesti oka i sa razvojem dijabetesnog makularnog edema i proliferativne dijabetesne retinopatije.

Ključne reči: dijabetesna retinopatija; makularni edem; katarakta; komplikacije dijabetesa; dijabetes melitus tip 2; glikemija; intraokularni pritisak; glikozilizirani hemoglobin; hipertenzija

Introduction

Diabetes mellitus is one of the most common metabolic diseases today. The clinical course is chronic and lifelong. It has vast socio-economic impact due to the high degree of morbidity, mortality and handicap it is causing [1, 2].

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Abbreviations

HbA1C – glycosylated hemoglobin
IOP – intraocular pressure
NDR – nonproliferative diabetic retinopathy
PDR – proliferative diabetic retinopathy
DME – diabetic macular edema
WESDR – Wisconsin Epidemiologic Study of Diabetic Retinopathy
DM – diabetes mellitus

Diabetes can cause a number of complications that affect a multitude of tissues and organs. The main causes of complications patients are experiencing are the changes in structure and function of blood vessels throughoutone organism.

In the time before the discovery of insulin, due to the much shorter lifespan of patients with diabetes, late complications could not develop and they were seldom diagnosed. The patients today live significantly longer and together with advancement in examination techniques, complications are detected much more often.

In the majority of patients, irrespective of the type and the quality of diabetes treatment, the first changes of the posterior segment of the eye start to appear 10 years after diabetes is diagnosed in the form of diabetic retinopathy [3, 4].

The main hallmarks of diabetic retinopathy are initial damage of small retinal blood vessels (diabetic angiopathy), followed by nonproliferative phase that progresses to proliferative phase of the disease in some cases [3]. Although nonproliferative retinopathy usually does not cause vision loss and not require treatment, macular edema must be treated. Unfortunately treatment is usually effective at stopping and sometimes reversing vision loss. Diabetic macular edema (DME) is the most common cause of vision loss among people with diabetic retinopathy. It can happen at any stage of the disease, although it is more likely to occur as diabetic retinopathy worsens.

According to the United States epidemiology data, 80% of type 1 diabetes and 40% of patients with type 2 diabetes show signs of retinopathy during the course of the illness [5, 6]. Diabetic retinopathy is the main ocular complication of diabetes and it is the main cause of visual impairment among working age population [7].

There are several known factor responsible for the development of diabetic retinopathy, duration of disease and blood sugar level being the most important ones. Diabetic retinopathy hardly ever occurs in the first 5 years of the disease or before puberty. The duration of diabetes is one of the most significant factors for the development of diabetic maculopathy and the progression from nonproliferative to its proliferative stage [7]. Proliferative phase of diabetic retinopathy is found in 15% of patients after 15 years and in 50% of patients with 25 years of history of diabetic retinopathy [8].

Regulation of glycaemia is another important factor to consider in the development of ocular complications of diabetes. Proper management of blood sugar levels reduces both the risk of diabetic retinopathy appearance and its progression to more severe proliferative stages [8]. It has been known that glycemic control started in the early stages of the disease can postpone or even prevent the occurrence of diabetic retinopathy. Increased serum level of glycosylated hemoglobin (HbA1C), as one of the most important diabetic markers, is related to worsening of the clinical course of diabetic retinopathy [7]. It is determined that its overall reduction decreases the risk of diabetic retinopathy and the risk of visual loss. In the same time, the need for laser photocoagulation therapy is lowered by 25% [9, 10].

Rheological characteristics of the blood, its viscosity and hematocrit and haemoglobin levels have strong influence on retinopathy development [11]. Arterial hypertension is very common in patients with diabetes. It is found that reduction of systolic pressure by 10 mmHg lowers the risk of retinopathy progression by 35% and the risk of visual impairment by 50% [9].

Vision loss due to diabetic retinopathy is sometimes irreversible. However, early detection and treatment can reduce the risk of vision-threatening complications.

The purpose of this prospective study was to investigate the onset and the course of diabetic eye diseases according duration of diabetes mellitus (DM), level of blood and ocular pressure and blood glucose level.

Material and Methods

This prospective study included 280 consecutively diabetic patients (diabetes mellitus type 2) at the Department for Medical retina, Eye Clinic, Clinical Center of Vojvodina between October 2015 and December 2016. According to the duration of diabetes mellitus patients were divided into 3 groups:

– Group I: 88 patients with diabetes diagnosis 5–10 years

– Group II: 112 patients with diabetes diagnosis 11–15 years

–Group III: 80 patients with diabetes diagnosis ≥ 15 years

All diabetic patients included in this study underwent complete ophthalmological examination: distance visual acuity testing using Snellen method, intraocular pressure (IOP) measurement with applanation tonometry, slit lamp examination of anterior and posterior segment of the eye in artificial mydriasis and optic coherence tomography (Stratus TD-OCT, Carl Zeiss Mediatec USA).

A full medical history included patient age, the time elapsed from diabetes diagnosis, current treatment of diabetes, presence of hypertension and glycemic control assessed by HbA1C measurement.

The classification of diabetic retinopathy was performed according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria. DME was defined as retinal thickening on optic coherence tomography (OCT) within 500 μm of the center of the macula; and/or hard exudates at or within 500 μm of the center of the macula; and/or a zone or zones of retinal thickening one disc area in size, any part of which is within one disc diameter of the center of the macula.

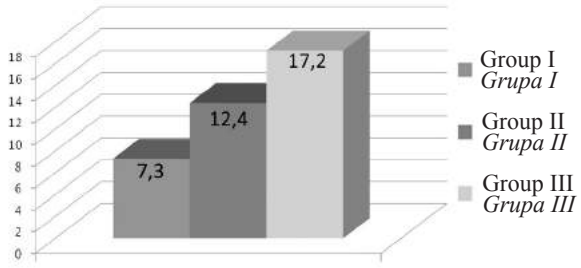

Figure 1. Mean duration of diabetes
Slika 1. *Prosečna dužina trajanja šećerne bolesti*

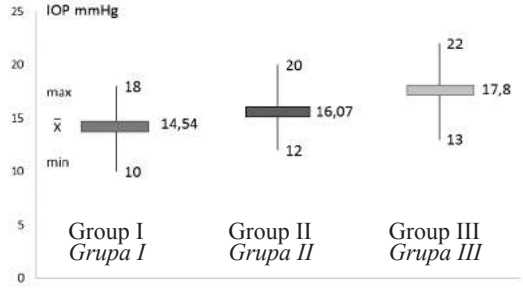

Figure 3. IOP values
Slika 3. *Visina intraokularnog pritiska*

Patients with other ophthalmic conditions (a history of cataract surgery, laser treatment, glaucoma, uveitis etc.) or systemic diseases that might have influenced the onset and course of diabetic eye disease were excluded.

Diabetic patients with identified preproliferative retinopathy or sight-threatening maculopathy were referred to further treatment and follow-up.

Results

A total of 280 patients were enrolled in this study, 160 (57.1%) females and 120 (42.9%) males. The mean age of patients was 63.5 years (SD±6.5, range 57-70 years).

Mean duration of diabetes was 7.3 years in group I, 12.4 years in group II and 17.2 years in group III **(Figure 1)**.

The average value of HbA1C was 6.58% in the group I, 7.64% in the group II and 8.29% in the third group of patients.

Assessment of HbA1C values between groups revealed statistically significant higher values of HbA1C in group III in comparison to group I (6.58 vs. 8.29, $p < 0.05$). Average values of HgA1C are presented in **Figure 2**.

IOP values among patients included in the study ranged between 10 and 24 mmHg. Average value in group I was 14.54 mmHg, in group II 16.07 mmHg and in group III 17.8 mmHg.

No statistically significant difference in IOP values was found among examined groups **(Figure 3)**.

Values of blood pressure higher than 140/90 mmHg were observed in 126 (45%) patients. Analyzed by groups, based on the duration of diabetes, 40 patients (45.45%) in group I, 48 patients (42.8%) and 42 patients (52.5%) had elevated blood pressure above border value. No statistically significant difference in the level of blood pressure among groups was noted. Blood pressure values in observed groups are shown in **Figure 4**.

Complications related to diabetes among all of the patients included in our study were: nonproliferative diabetic retinopathy (NDR) in 48.5%, proliferative diabetic retinopathy (PDR) in 25.7% and DME in 22.5% of patients.

Nonproliferative diabetic retinopathy was present in 24 patients (27.27%) in group I, PDR in 16 patients (18.18%) and DME in 8 patients (9.1%). In group II NDR was found in 56 patients (50%), PDR in 24 patients (21.4%) and DME in 31 patients (27.67%). In the same time, there were 56 patients (70%) with NDR, 32 patients (40%) with PDR and 24 patients (30%) with DME in the group III. Analysis of diabetes related complications with duration of diabetes and the degree of regulation of blood sugar (HbA1C) revealed significantly higher number of complications in group III compared to other two groups of patients **(Figure 5)**.

Cataract was present in 104 patients (37.1%). Comparison of groups of patients, based on diabetes duration, showed cataract in 27.27% of patients in group I, 35.7% of patients in group II and 50% of

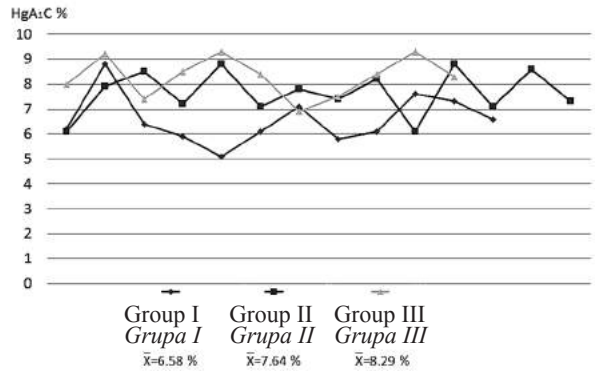

Figure 2. HgA1C values
Slika 2. *Vrednosti glikoliziranog hemoglobina*

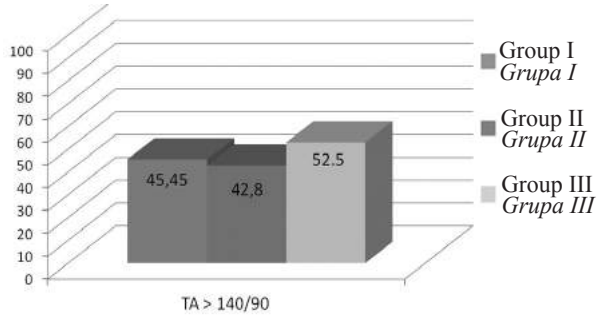

Figure 4. Blood pressure values
Slika 4. *Visina krvnog pritiska*

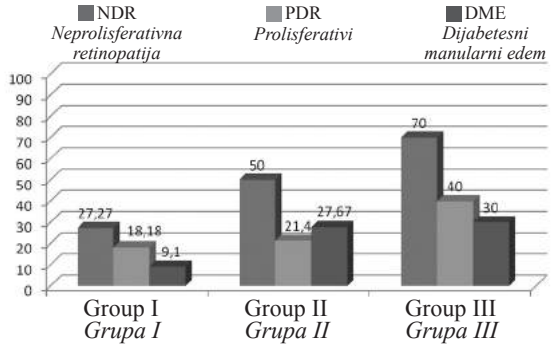

Figure. 5. Rate of diabetes complications
Slika 5. *Stopa komplikacija u vezi sa šećernom bolešću*

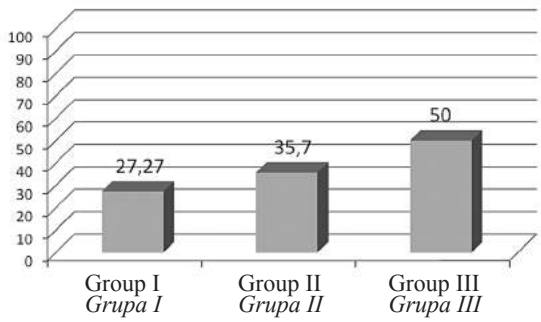

Figure. 6. Cataract and diabetes
Slika 6. *Udruženost katarakte i šećerne boelsti*

patients in group III. There was statistically significant difference found between group I and group III $p < 0.05$) **(Figure 6)**.

Discussion

Diabetic retinopathy (DR) is one of the leading causes of visual problems and blindness worldwide. Over the last decade the DR rate increased over 89%, despite tremendous advances in treatment of risk factors, such as high blood glucose level, blood pressure control etc. [12].

Recent estimates show that serious visual impairment and sight threatening diabetic retinopathy have 5% of US adults. Unfortunately, there is no evidence of diabetic eye diseases in our country.

Though diabetes rates continue to rise, blood glucose control has improved over the past two decades and fewer people with diabetes are going undiagnosed.

Epidemiological studies have shown that major predictors of sight threatening complication in diabetic patients are the presence and severity of retinopathy at a patient's first retinal examination. Our study found NDR in 48.5% and PDR in 25.7% at the first examination.

Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) found that people with diabetes diagnosed before 30 years of age and without retinopathy at baseline, only 0.4% progressed to PDR over 4 years. In contrast, patients with early retinopathy at baseline progressed to PDR over 4 years in 9% [13]. The average age of diabetic patients included in our study was 63.5 ± 6.5 years (57–70 years).

Many epidemiological and clinical studies documented the higher prevalence of cataract in diabetic patients [14, 15]. Association between diabetes and cataract is shown in the Beaver Dam Eye Study, the Blue Mountains Eye Study and the Visual Impairment Project [16, 17].

The total number of patients with cataract in our study was 104 (37.1%). There were 27.27% diabetic patients with cataract in group I, 35.7% in group II and 50% in group III ($p<0.05$).

The WESDR documented a direct relationship between HbA1C and cataract [18]. Our study also found increased number of cataracts in diabetic patients with elevated HgA1C (group III, $p<0.05$).

Patients with diabetes also tend to get cataracts at a younger age and have them progress faster. The risk of developing cataract in diabetic patients increases with increasing duration of diabetes [19]. Our study also showed higher incidence of cataract in patients in group III (patients with diabetes diagnosis ≥ 15 years) comparing to patients in group I (patients with diabetes diagnosis 5–10 years) ($p<0.05$).

Wisconsin Epidemiologic Study of Diabetic Retinopathy have shown cumulative incidence of DME in 29% of patients with diabetes after 25 years [20].

Williams et al., 10 years after initial diagnosis of diabetes type 2, reported DME in 13.9% of patients on oral hypoglycemic therapy and in 25.4% of patients treated with insulin [21].

The incidence of DME in our study was 27.67% in group II (11–15 years after initial diagnosis of diabetes) and 30% in group III (diabetes diagnosis ≥ 15 years).

Numerous studies have shown that proper control of glycaemia decreases the risk of diabetic retinopathy development and that the values of HbA1C in the range from 6.5% to 7% are optimal and something to strive for. Patients with values of HbA1C from 7% to 8% require more frequent follow-ups and additional regulation of the systemic disease. Values over 8% indicate poor regulation of systemic DM and cases with HbA1C over 9% are strongly correlated with rapid progression of microvasculature complications [22–24].

The average value of HbA1C found in our study was 6.58% in the group I, 7.64% in the group II and 8.29% in the third group of patients. Statistically significant higher values of HbA1C were found in patients with average duration of the disease longer than 17.2 years (group III), in comparison with the patients in first two groups.

Epidemiological studies and clinical research indicate that arterial hypertension is important risk factor for the development of DR in patients with DM [22]. Elevated blood pressure causes mechanical damage (distension) of endothelial cells of blood vessels stimulation additional release of vascular endothelial growth factor.

Around 40% of patients with type 2 diabetes are hypertensive, the proportion increasing to 60% by the age of 75. In the United Kingdom Prospective Diabetes Study Group a 10 mm Hg fall in systolic blood pressure and a 5 mm Hg fall in diastolic blood pressure was associated with a 47% reduction in risk of doubling of visual angle at 9 years [25, 26]. In the years after diagnosis of type 2 diabetes the incidence of hypertension is higher than in the age matched general population.

In our study 128 (45.7%) of patients with type 2 diabetes were hypertensive. There was higher incidence of PDR and DME in group III comparing to patients in group I ($p<0.05$).

The relationship between diabetes and glaucoma currently remains controversy. Several population-based studies have shown that patients with diabetes and elevated blood glucose level were associated with higher IOP, and at risk for primary open angle glaucoma development [27, 28]. Our study showed that there was no significant difference in IOP in diabetic patients according to the duration of DM. The average IOP was 14.54 mmHg in group I, 16.07 mmHg in group II and 17.8 mmHg in group III. There was increased HbA1C level in patients with diabetes diagnosis $\geq$ 15 years, comparing to other groups of diabetic patients ($p<0.05$), but there was no statistically significant correlation between IOP and HbA1C.

Treatment for ocular complications of diabetes is continuously evolving. Clinical and experimental studies show the benefit of intravitreal anti vascular endothelial growth factors medications, corticosteroids and numerous oral supplements [29–31].

Conclusion

Our study confirmed that the risk of developing preproliferative retinopathy, diabetic macular edema and diabetic cataract increases with increasing duration of diabetes. There is significantly higher incidence of preproliferative retinopathy and diabetic macular edema in diabetes accompanying with hypertension. Increased serum level of glycosylated hemoglobin is related to worsening of the clinical course of diabetic eye diseases, developing diabetic macular edema and preproliferative retinopathy.

Concerning the results of many previous epidemiological and clinical studies and results from our study in evaluation of factors contributing to the onset and to the course of diabetic eye diseases, people with diabetes should get appropriate screening programme. Patients with diabetes require regular follow up with primary care physicians to optimize their glycemic, blood pressure and lipid control to prevent development and progression of diabetic retinopathy and other diabetes-related complications.

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COLONOSCOPY IN COLORECTAL CANCER DIAGNOSTICS – THE MOST FREQUENTLY ASKED QUESTIONS AND DILEMMAS

KOLONOSKOPIJA U DIJAGNOSTICI KOLOREKTALNOG KARCINOMA – NAJČEŠĆA PITANJA I DILEME

Biljana KUKIĆ

Summary
Colorectal cancer is the third most frequent illness of all carcinomas with an increase in the incidence of colorectal cancer in highly developed countries. 75% of patients with colorectal cancer are older than 65 years. It is believed that 66–75% of colorectal cancer could be avoided through healthy lifestyle. 75% of colorectal cancer arise from adenomatous polyp but more than 90% of adenomas will not progress to carcinoma. Colonoscopy has become the preferred method for evaluation of lower digestive symptoms and detection and treatment of colon pathology, and is considered to be the gold standard for colorectal cancer screening and surveillance. Aim of this paper is to resolve some dilemmas and answer the most frequently asked questions from primary care physicians about colonoscopy such as indications, contraindications, the role of colonoscopy in colorectal cancer screening, how often colonoscopy should be repeated, why it is so important to prepare colon and patients for colonoscopy and what type of complications can happen.
Key words: Colonoscopy; Colorectal Neoplasms; Mass Screening; Diagnosis; Signs and Symptoms

Sažetak
Kolorektalni karcinom je na trećem mestu po učestalosti obolevanja od svih karcinoma uz porast incidencije u visokorazvijenim zemljama, 75% pacijenata sa kolorektalnim karcinomom stariji su od 65 godina. Uz zdrav način života, veruje se da se kolorektalni karcinom može izbeći u 66–75% slučajeva. U 75% slučajeva, kolorektalni karcinom nastaje iz adenomatoznog polipa, ali više od 90% adenomatoznih polipa ne napreduje u karcinom. Kolonoskopija je postala metoda izbora za procenu simptoma donjeg digestivnog trakta u otkrivanju i lečenju patologije kolona i smatra se zlatnim standardom za skrining i praćenje kolorektalnog karcinoma. Cilj rada je da se odgovori na najčešća pitanja i dileme koje se u vezi sa kolonoskopskim pregledom i koje se javljaju u praksi − kao što su indikacije i kontraindikacije za izvođenje intervencije, uloga kolonoskopije u skriningu kolorektalnog karicnoma, intervali ponavljanja pregleda, važnost pripreme bolesnika i kolona za pregled, dužina trajanja intervencije i njene komplikacije.
Ključne reči: kolonoskopija; kolorektalni karcinom; skrining; dijagnoza; znaci i simptomi

Introduction

Colorectal cancer is the third most frequent of all carcinomas with an increase in the incidence of colorectal cancer in highly developed countries. 70% of patients with colorectal cancer are older than 65 years [1, 2]. It is believed that 66–75% of colorectal cancer could be avoided through healthy lifestyle [3]. 75% of colorectal cancer arise from adenomatous polyp but more than 90% of adenomas will not progress to carcinoma [4].

Colonoscopy is the gold standard in the diagnosis of colorectal carcinoma (CRC) and, thanks to biopsies and polypectomy, it has enabled the extension of knowledge about the nature of CRC. Resection of benign adenomas prevents the formation of CRC by interrupting the polyp-cancer sequence (it takes 10–15 years for neoplastic transformation of adenoma into the cancer) [3, 5]. The success of colonoscopy depends on the sex, age, obesity, preparation of the colon, previous pelvic surgeries, complicated diverticulous diseases and earlier peritonitis.

Indications and Contraindications

Symptoms that are considered essential for the diagnosis of CRC are rectal bleeding, change in stool rhythm, abdominal pain, weight loss, diarrhea and constipation. The meta analysis examining the association of symptoms and CRC has shown that only rectal bleeding and weight loss are related to CRC, while there were insufficient evidence for other symptoms. A person losing weight has a 3x greater risk that the cause of a problem is CRC compared to those who have no weight loss, but the problem is that weight loss occurs very often as a symptom of other diseases [6]. Cox et al. analyzed the risk of CRC in relation to the symptoms and showed that the risk of CRC was elevated in men with a positive family anamesis for gastrointestinal carcinoma (1.5x), anemia (3.3x), rectal bleeding (27x in the middle age od life), abdominal pain (6.8 x), loss of appetite (2.2x), weight loss (4.1x) or change in the discharge rhythm (2.3x). Younger men with rectal bleeding and abdominal pain have a higher risk of having CRC. Symptoms related to CRC in female sex are

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Abbreviations
CRC – colorectal carcinoma
IBD – inflamamatory bowel disease
BBPS – Boston Bowel Preparation Scale

similar and a significant association between the three factors has been found - weight loss, abdominal pain and rectal bleeding and CRC, and this association is more pronounced in the younger age [7].

Indications for colonoscopy are divided into diagnostic and therapeutic. Diagnostic indications include: screening for persons with an average risk for CRC, CRC-controlled patients, persons with adenomas, family anamnesis of adenomatous polyps or CRC, hereditary nonpoyposis CRC syndrome, patients with endometrial or ovarian carcinoma under 50 years of age, long-term unexplained diarrhea and bleeding from the digestive tract, a positive fecal occult blood test, sideropenic anemia, hematohesia if the cause of bleeding cannot be found by anoscopy and sigmoidoscopy, melena if the cause of bleeding from the upper gastrointestinal tract parts is excluded, a pathological finding on radiological contrast image of colon, or another diagnostic method indicating a defect in filling, squatting or thinning of the wall of the bowel, evaluating of the colon after polypectomy during sigmoidoscopy, diagnosis of inflammatory bowel disease (IBD), determination of extension and monitoring of dysplasia in long-term IBD, intraoperative colonoscopy – the alignment of the previously removed polyps, for finding bleeding site or detection site of rest lesion requiring surgical resection as well as inability to perform preoperative colonoscopy. It should be noted in particular that the colonoscopy is not indicated in patients with chronic, stable spastic colon, acute diarrhea, metastatic adenocarcinoma of unknown primary site in the absence of colon-related symptoms when the intervention will not affect the therapeutic plan as well as in the case when melena is caused by bleeding in the upper part of gastrointerstinal tract. Like any invasive method, colonoscopy has contraindications that can be relative and absolute. Absolute contraindications are: acute peritonitis, perforation of the intestine, acute diverticulitis, fulminant colitis, toxic megacolon, suspected obstruction of the intestine and non-cooperative patient, while relative contraindications are: acute myocardial infarction, serious heart rhythm disorders, late pregnancy, recent colorectal surgery and inadequate preparation of patients for colonoscopy [8–13]. Some authors believe that these guidelines have suboptimal sensitivity from individual patients with CRC. If the overall prevalence of CRC is 4.4% and the colonoscopy is performed in 26% of people without the right indication-with a prevalence of CRC of 1.9%, this means that there is a chance of 1: .54 to overcome the malignancy of the colon in those individuals who do not belong to any of the above-indicated indications for colonoscopy. Colonoscopy in order to exclude colorectal cancer is performed due to the large number of different symptoms associated with the colon [14].

What is the Role of Colonoscopy in Colorectal Cancer Screening?

Colonoscopy is considered the most effective CRC screening method for people older than 50 years. However, more and more evidence suggest that this method is less effective in preventing proximal versus distal carcinoma. It is believed that the cause of these differences is in the biological difference between the polyp and carcinoma of the left and right colon, unrecognized or incomplete resected advanced adenomas and other precancerous leasions (flat lesions and serrated adenomas), lesion localized behind colonic haustras, inadequate colon preparation and insufficient experience of endocopist [15]. Prospective and observational studies have reported a significant reduction in incidence and mortality from CRC in screening studies up to 67%. Colonoscopy has a sensitivity of 98% and a specificity of 99% for lesions greater than 6 mm. There is still insufficient evidence to suggest the correlation between colonoscopy and the risk of different types of precancerous lesions (different biological pathways of the CRC), which primarily relates to serrated adenomas as important precursors of the right colon carcinomas [15, 16]. A study that encompassed 35,918 of patients with screening colonoscopy detected 2,544 advanced neoplasia (7.1%) and showed that age, sex, family history of CRC, smoking and body mass index were independent risk factors for advanced colorectal neoplasia, which is of great use in their diagnosis in asymptomatic persons [17]. In tandem studies, screening colonoscopy reported 6-12% of undetected large polyp and about 5% of CRC at initial colonoscopy, so it is necessary to provide good quality colonoscopy, which implies good preparation of colon and endoscopists with sufficient experience [18]. A large problem in the use of colonoscopy in screening is a low response of the people with positive occult blood test to this intervention (preparation is needed, fear of anesthesia, shame ...). An analysis of 161 patients in the Institute of Oncology of Vojvodina proved that 70.8% of the examinees have fear of colonoscopy due to findings (45.8%), 9.3% had fear of pain during the examination, and 28% had a combination of these two reasons. Only 8.4% had a delay in colonoscopy due to inconvenience. Women are statistically more commonly scared than men 75.7% vs 59.1% [19]. CRC colonoscopy screening is applied to both sexes starting from 50 years of age. A Bavarian study that analyzed 625.918 outpatient colonoscopies in order to determine the risk of advanced adenomas below the scaling margin showed that the male sex has a higher risk of advanced adenomas between age 40–79 compared to women of the same age, and there are suggestions that the CRC screening in male population should be conducted earlier, which could increase the detection of asymptomatic pre-neoplastic and neoplastic colon lesions [20].

How often should Colonoscopy be Repeated?

The question arises as to the risk of CRC after a negative finding of a screening of the colonoscopy. Canadian studies showed 30–40% reduction in CRC inci-

dence after a negative review and a reduction in the risk of developing this disease for 10 years, which depends on patients, endoscopists and factors related to the procedure itself, is subject to regional variations and, consequently, differences in incidence of CRC after a negative colonoscopy [21]. According to current recommendations in a population with a common risk, screening colonoscopy should be repeated after 10 years, although some studies indicate that the negative screening colonoscopy has a protective risk of CRC for 20 years. Repetition of colonoscopy after 5 years of negative colonoscopy showed that ≤3% of the subjects had advanced adenomas and the results of the studies of flexible sigmoidoscopy and colonoscopy with polypectomy showed that these methods had a protective effect for 10–16 years and more. Colonoscopy in shorter intervals is not indicated , increases the risk of complications of intervention and unnecessarily financially burdens the health system. Patients with negative colonoscopy and rectal bleeding may be referred for re-examination in a shorter interval. Guaiac fecal occult blood test (gFOBT) in the first 5 years after a negative colonoscopy is not recommended due to a small positive predictive value. 2–7% of patients with CRC have a synchronous tumor at the time of diagnosis, which is difficult to diagnose in patients with obstruction and inadequately prepared colon, so in the follow-up the first colonoscopy is recommended within one year after surgery or within 6 months after surgery in cases when total colonoscopy was not performed before operation [22]. 30–40% of the patients have relapses of colorectal carcinoma after curative resection, and colonoscopies are conducted for the purpose of monitoring and detection of relapse at an early stage. However, 10–20% of the CRC relapse occurs locoregionally and is not visible endolumenally. The American Cancer Society, the American Gastroenterology Association (AGA) and the United States Multi-Society Task Force on Colorectal Cancer recommend that the first colonoscopy be done in the first year after surgery, and if the finding is correct, the next should be done after 3 and then every 5 years [23, 24].

Why is good Colon Preparation Important?

Each colonoscopic finding should include a description of the preparation of a colon based on the ability of the endoscopist to visualize the mucosa after the residual stool and fluid removing. It is marked as excellent, good, central, poor and unsatisfactory. The American Society for Gastrointestinal Endoscopy/American College of Gastroenterology (ASGE/ACG) Quality Indicator Task Force recomanded that the preparation of the colon may be considered adequate if polyps greater than 5 mm can be seen. Currently, the most widely used Ottawa Bowel Preparation Scale (OBPS), Boston Bowel Preparation Scale (BBPS) and Aronchick (Aronchick Bowel Preparation Scale - ABPS) are the scales of preparation that include the amount of suction content that can be removed during the examination and the quality of the preparation is based on the visualization of the mucosa after the aspiration of the residues in the lumen of the colon. If the preparation is inadequate colonoscopy should be repeated within a year [16]. In the wider use is the BBPS, which implies a score system (0–3): 0 = unprepared colon so that mucosa is not visualized, 1 = part of the mucosa is visible, but the parts of the mucosa of the colon are not 2 = small amount of stool or liquid content, but the mucosa of the colon is well visible 3 = complete mucosa is clearly seen without the presence of residues. Each part of the colon (segment score) ranges from 0 to 3, and a total score of 0–9 is obtained, so that the mininal score is in the unprepared colon 0 and in the ideal preparation 9 [25]. Inadequate preparation of the patient statistically significantly extends the examination time and increases the possibility of an error. In a large European study on 5832 patients, the cecum intubation was achieved in 90% of cases who were well prepared and in only 71% of those who were poorly prepared for examination. Also, poor preparation of the patient affects the prolongation of the time needed to reach the cekum and the average time is 11–16 minutes. The good preparation of the colon is an independent factor associated with an incomplete colonoscopy [26, 27]. The percentage of undiagnosed adenomas of all sizes in suboptimal colon preparation is high and ranges from 15–32% in repeated tandem colonoscopies, especially in those in whom at least one adenoma was diagnosed at the first colonoscopy (36% versus 20% for those who had negative findings). For advanced adenomas greater than 10 mm and histologically advanced lesions 1–8% were recorded, but difference in the number of undiagnosed polyps between the left and right columns was not recorded [28, 29]. If the preparation is inadequate, it is necessary to repeat colonoscopy within a year. In the case of medium but adequate preparation, when verified adenomas are smaller than 10 mm of colon, colonoscopy should be considered within the next 5 years. The large number of endoscopists in the case of suboptimal preparation recommend repeating the review in a shorter interval (3–5 years), regardless of the presence or absence of polyps. The time interval to the next colonoscopy is determined based on the preparation of the colon at the initial examination [16, 30]. Lebwohl et al. reported on the repeated colonoscopies after 3 years of initial with inadequate preparation 27% of undiagnosed adenoma [28] and Chokshi et al. concluded that inadequate preparations cannot be seen at least one adenoma in 33.8% of cases [31]. The suboptimal preparation extends the time of examination and reduces the number of detected polyps. It is considered that the number of inadequate examinations that require repetition of examinations should not exceed 10% annually. Endoscopists who exceed this percentage should re-evaluate the patient's protocol preparation, patient education, the choice of laxative and the route of administration. For good preparation of patients the interval between the last dose of laxative and the start time of the endoscopy are of great importance because the quality of the preparation decreases with the extension of this interval which is particularly relevant to the right colon. It is optimal that the dose of a laxative dividing, which means that the second dose is taken on the day of examination, and for procedures that are performed in the afternoon,

it is recommended to take the entire dose in the early morning on the day of examination. For interventions in the analgesia it is recommended that the patients do not take anything on the mouth for 2 hours before examination and the amount of contents in the stomach (<25 ml) does not depend on how the patient is prepared [16, 32].

How Long does Colonoscopy Last?

More detection of neoplastic lesions has been demonstrated if the time of colonoscopy drawing is ≥6 minutes. Six and more minutes is the time to draw the device in normal colonoscopies. Of course, each colonoscopy does not require 6 minutes to draw the device because the colon differs in length and in the conditions of good preparation of the column and non-linear haustral markers can be done in high quality and if the time of drawing the device is less than six minutes [16].

What are the Colonoscopy Complications?

Perforation of the colon is moste dramatic complication of the colonoscopy with an incidence of 0.04–0.9% for diagnostic and 0.06–0.7% for therapeutic procedures. Lately, the number of perforations is smaller, which is associated with a better endoscopic technique and the equipment used. Perforation is a complication caused by pressure of the instrument's tip, formation of the loop during examination, after the biopsy, after the polypectomy, or the dilation of the colon stent. Perforations during diagnostic colonoscopy are more common in the sigmoid colon and after therapeutic procedures in the right column. Particular risk of perforation is in incoperabile patients, those with poor bowel preparation, diverticuloses, ischemic colitis and obstruction of the colon. Although age is not an independent risk factor for the perforation, the female sex is. Perforation occurs during or immediately after the intervention, and about 5% of perforations have a fatal outcome. Population studies have shown that the risk of perforation for all colonoscopies regardless of indication is 1:500. If the colonoscopy is performed in screening, the risk of perforation is 1/1000, because the patients are in good shape and generally do not have other colon diseases (severe colitis, ischemia, post-surgical changes, contacts, serious diverticulosis ...) [16]. The incidence of perforation in the diagnostic colonoscopy is 0.04% and in the therapeutic maximum of 0.02%. Reported perforation rates in individual stadies range from 0–0.86% [33]. Bleeding is the most common complication of the colonoscopy with an incidence of 0.02–0.03% for diagnostic and 0.31–2.7% for therapeutic procedures. Bleeding after polypectomy is most often due to an inadequate balance between thermal and transsective forces, most commonly occurs immediately after intervention but can occur within 2 weeks after polyp resection and can be massive. Potential risk factors for the occurrence of bleeding after polypectomy are polyps above 2 cm especially large succulent or pendicular with a thin well-vascular pedicle, elderly life and coagulation disorders. The largest number of bleeding after polypectomy spontaneously stops and does not require any intervention [16].

Conclusion

Under conditions of good preparation of the colon (more than 90% of mucosa is visable) with intubation of cecum and according to current recommendations, colonoscopy in persons with usual colorectal cancer risk should be repeated every 10 years. It must be taken into account that the reduction of the incidence of colorectal cancer within the screening does not have to correspond with the reduction of mortality. Carcinomas resulting from premalignant lesions that can be detected and resected may be the ones with the slowest progression and, therefore, are more curable. For this reason, mortality associated with screening may be greater than the incidence (indicating poor protective power of the screening method). On the other hand, some tests may have greater sensitivity to early carcinoma than to polyps, so their impact on colorectal cancer incidence is smaller than the impact on mortality from this disease. The results of prospective and retrospective studies have confirmed that sigmoidoscopy and colonoscopy, which were performed for either diagnostic reasons in screening or monitoring, statistically significantly reduce incidence and mortality from colorectal cancer, and meta-analysis proved that in comparison with the so-called non interventional group colonoscopy decreased the colorectal cancer mortality by 57%, sigmoidoscopy by 40% and guaiac fecal occult blood test by 18%.

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THE 21ST CENTURY – THE ROLE OF THE PHARMACIST IN THE HEALTHCARE

21. VEK – ULOGA FARMACEUTA U ZDRAVSTVENOJ ZAŠTITI

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Summary
The roles of the pharmacist were transformed throughout the history and modern pharmacist as we know now beside providing products and playing in optimization of medicines has a crucial role in ensuring the efficacy and safety of applied drugs. A better life quality, global health and safety is the major goal of the 21st century and to achieve that great span of roles pharmacy profession involves now. The paper highlighted new roles of the pharmacist today in order to better understand the transition of the pharmacy profession.
Keywords: Pharmacists; Delivery of Health Care; Public Health; Professional Role; Pharmaceutical Services; Health Promotion

Sažetak
Kroz istoriju, uloga farmaceuta se menjala i farmaceut kakvog danas poznajemo, pored davanja, pravljenja i optimizacije lekova prema preskripciji, ima značajno mesto u obezbeđenju sigurne i efikasne primene propisanog leka. U skladu sa osnovnim postulatom 21. veka o boljem kvalitetu života, zdravlju i bezbednosti, proširuje se profesionalna uloga farmaceuta u pružanju zdravstevne zaštite i javnom zdravlju. Radi boljeg razumevanja farmaceutske profesije danas, u radu su istaknute savremene uloge faramceuta.
Ključne reči: farmaceuti; pružanje zdravstvene zaštite; javno zdravlje; profesionalna uloga; farmaceutski servisi; promocija zdravlja

Introduction

Throughout the history, the roles of the pharmacist were modified. Before the 18th century the pharmacist was responsible for the treatment and prescribed therapy for diseases and production of medicines. The development of the pharmaceutical profession has started since the beginning of the 18th century and some of roles modern pharmacists as we know now still play [1]. With the improvement of the pharmaceutical industry the pharmacist took a significant part in the drug development and manufacture of the finished dosage form. From the second part of the 20th century, important roles of the modern pharmacist became the quality control and the selection of medicines based on the cost-effectiveness, the development of the improved drug delivery systems, the distribution of medicines to the private and public pharmacies as well as the control of the drug availability, the price and the drug use [2, 3]. In some developed countries a collaborative agreement between one or more doctors and pharmacists about making decision, selection, application, testing and monitoring of drug use exists [4].

According to the Alma Ata Declaration, that promotes "Health for All" and the "Universal Health Coverage", the promotion and protection of health are crucial for people's well-being and the sustainable socio-economic development. All above mentioned contribute to a better life quality, global health and safety [5–7]. In the 21st century, the professional roles of the pharmacist are expanded and they participate more than previously in the healthcare, clinical services as well as in the optimization of the therapy through the counseling with doctors, patients and other healthcare workers. Therefore, the pharmacists have an important role in the public health chain [4]. According to that, the modification of the undergraduate pharmaceutical education is necessary in order to ensure the successful cooperation between pharmacists and all other health professionals in the health promotion and disease prevention [8].

Also, in the modern society the pharmacist plays an important role in public health through the counseling about high blood pressure, diabetes, overweight and obesity, elevated blood lipid levels, physical activity, and smoking in order to reduce the risk and incidence of the mass non-communicable diseases [3, 8, 9]. Today, pharmacists also play a significant role in supporting people with mental illnesses [10] as well as

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Abbreviations
OTC – over-the-counter
EDCs – endocrine disrupting chemicals
NBCDs – non-biological complex drugs
EMA – European Medical Agency
GMP – good manufacturing practice

in the screening for immunization [4, 8]. The development of drugs market as well as market of dietary supplements, herbal remedies and herbal supplements requires greater involvement of pharmacists through counseling about drug-nutrient and drug-food interactions in order to ensure the efficacy of the applied drug and to reduce the possibility for harmful effects to health [11]. Regarding a large number of medicines sold directly to consumer without the prescription (over-the-counter (OTC) drug) the pharmacists have to provide comprehensive professional information and ensure the safe use of OTC medicines [12]. The modern role of pharmacists also involves the professional care about the possible negative effects of the environment pollution to the human health. The development of new food technological processes, consumer goods and cosmetics production, as well as the synthesis of new materials that are used in everyday life requires the increase of the professional awareness especially observing endocrine disrupting chemicals (EDCs) [13].

Pharmaceutical sector is covered with wide number of regulations, codes and guidelines controlling the process of research and development, manufacturing and drug commercialization. The role of the pharmacists of the 21st century is to continuously apply, follow and install current standards and roles necessary for all fields of pharmacy. One of the most recognized standards for control and management of manufacturing and quality control testing of pharmaceutical products is the Good Manufacturing Practice (GMP) [14].

Having in mind the great span of roles of the pharmacist of the modern society newer roles will be explained below in detailed in order to better understand the transition of the pharmacy profession in the 21st century.

The Development of the Improved Drug Delivery System

The progress in pharmaceutical sciences and manufacturing techniques gave rise to a new class of medicinal products with complex macromolecular (nanoparticulate) structures, the so-called non-biological complex drugs (NBCDs). It is directed towards development of drug delivery systems to improve disease-specific targeting, to control drug release rates and/or to produce a pharmaceutical formulation suitable for clinical use. One of the strategies to get NBCDs is encapsulation of the active substance in the aqueous phase of a liposome, or incorporation or binding to the lipid components. Therapeutic equivalency of such a unique formulated nanovector for drug delivery is not easy to achieve. The question is what regulatory criteria should be used for assessing and approving future NBCDs and their follow-on products. For example, in the medical community there had been multiple clinical observations that Lipodox® did not appear to be as effective as Doxil® in recurrent ovarian cancer patients [15, 16]. A comparison study of liposomal amphotericin B products with different or the same chemical compositions, using different methods of production, showed variations in size, and exerted different *in vitro* and *in vivo* toxicities along with reduced efficacy [17]. These results underscore the importance of establishing appropriate bioequivalence testing for liposome products to ensure uniformity of their therapeutic index, since the standard development programme applied to demonstrate therapeutic equivalence for small, well-characterized molecules does not apply to complex drugs. Based on the experience with liposomal formulation of doxorubicin and amphotericin B it seems like a prospective clinical comparison of follow-on products is warranted to determine equivalency.

The European Medical Agency (EMA) considers requesting a physicochemical comparison between innovator and copy as well as a bioequivalence study with pharmacokinetic comparisons in patients of free drug and drug encapsulated by the liposomes. The necessity of a clinical efficacy trial is decided upon on a case-by-case basis [18]. EMA also considers which pharmaceutical data is needed as evidence of product comparability between test and reference or after changes to a liposomal product, to support comparative safety and efficacy, necessity of pre-clinical and clinical studies and circumstances which may allow to waive certain studies and consider the design of relevant *in vivo* non-clinical studies and the potential role for *in vitro* models [19].

The Personalized Therapy

Knowing what Hippocrates once said: "It is far more important to know what the person who is affected is like than what is the disease from which the person is ill" it would be difficult not to mention two extremely important areas that are intensively developing: pharmacogenetics and pharmacogenomics. By the end of 2003, the Human Genome Project provided new opportunities for the use of genetic information in the individualization of therapy [20]. In 1892, British physician Sir William Osler recalled the importance of genetic variability in medicine: "If there is no such variability among individuals, medicine would be a science like any other, and not a skill." Pharmacogenomics examines the impact of human genome polymorphisms in response to drugs, while pharmacogenetics is narrower area that studies the impact of individual, candidate genes in response to drugs [21, 22]. Given the significant technological advancement in the next few years we can expect that pharmacogenomics will be common in clinical practice, characterized as personalized medicine. Personalized medicine will become part of everyday clinical practice in which the genetic characteristics of patients will be used in risk prediction, diagnosis, therapy optimization, maximum

effectiveness and minimal side effects of the drug [23]. Pharmacists who synthesize new drugs will play a key role between geneticists and doctors in order to optimize the therapy in personalized medicine. As experts in drug therapy, pharmacists are in a unique position to shift the boundaries of pharmacogenetics both in research and in clinical practice [24].

The Prevention and Control of Chronic Non-Communicable Diseases

The prevention and control of chronic non-communicable diseases (cardiovascular diseases, cancer, chronic respiratory diseases and diabetes mellitus) is the major public health challenge and requires integrated action of all relevant factors [25]. The pharmacist's role is clearly recognized [26]; and it is an integral part of numerous initiatives that consider common as well as specific approaches to each individual disease [27, 28]. In case of diabetes, the European Forum of National Pharmaceutical Associations in collaboration with the World Health Organization developed the Programme "The role of the pharmacist in diabetes care" (Pharmadiaβ) [28]. The main activities relate to promoting of healthy lifestyles; early identification of people with diabetes; education about self-care and self-monitoring; dispensing medicines, medical devices and other health products; identifying and resolving drug-related problems [28]. Pharmadiaβ is the basis for national and regional programmes and it is carried out in accordance with Ethical Standards defined in the document Good Pharmacy Practice in Community and Hospital Pharmacy Settings. Finally, the Programme is an example how pharmacists can develop their new professional role.

The Phytotherapy

The usage of plants for prevention and treatment of various diseases is constantly increasing in modern society. Numerous herbal remedies and plant-based dietary supplements are available on the market, so this area today is an important and often underestimated part of health services. Therefore, the World Health Organization has created a strategy related to traditional medicine [29]. The goals of the strategy are development of safe, efficient and high quality herbal remedies through the control of research in this field and the continuous expansion of knowledge, but also through quality assurance standards for both raw plant material and herbal remedies. Also, an important part of the strategy is the integration of herbal medicines into one's health system. This would increase the availability of such products, but also ensure their rational use. So, the rational phytotherapy would be the phytotherapy of the 21st century. Consumers of herbal medicines should be warned to obligatory report the usage of herbal remedies to their physician or pharmacist, especially in the case of the concomitant use of a conventional drug because of the risk of possible adverse interactions. Also, the involvement of wider community (e.g. media, school) should be included in the implementation of these preventive measures together with health care professionals, in order to rise up the awareness about the safety of herbal remedies usage and treatment in general. On the other hand, health care professional with pharmacist being at the forefront of should constantly improve their knowledge on herbal remedies [30].

The Application of Antioxidants in the Therapy and Protection

In the last decades there is an increased interest of scientists in isolation of numerous biologically active compounds from different natural sources, for production of dietary supplements or functional food. Many recent publications, in the field of pharmacy, medicine and biology, are focused on clinical trials investigating their efficacy, but also analyzing their bioavailability and influence on a patient individually, taking into consideration specific physiology or enzymes in every organism. Since oxidative stress is considered to have a crucial role in pathophysiology of many degenerative diseases, pharmacists recognized the importance of certain natural antioxidants in therapy or prevention. For example, dietary intake of plant phenolics, widely present in fruits, vegetables and many other natural sources, has been associated with reduced risk of cardiovascular diseases, diabetes, obesity, cancer [31]. *In vivo* assessment of efficacy of these compounds should also consider the individual metabolism as well as their final concentration in target tissues and cells in human body [32]. It is proven that polyphenols can alter the composition and activity of the gut microbiota, which can have great influence on further metabolic path [33]. There is also growing number of studies concerning incorporation of phenolics into nanocarriers, liposomes and cyclodextrines, because of many advantages of these formulations, like physical and chemical stability, better bioavailability of active substances and increased concentration in target tissues [34].

Drug-Nutrient and Drug-Food Interaction

The progress of medicine and pharmacology has led to the development of a large number and types of drugs for various diseases and conditions. By definition drugs are chemical compounds that affect life processes. However, the development of the food and pharmaceutical industry has resulted in the existence of a huge market for dietary supplements that they have been using with or without the advice of a physician or pharmacist. Dietary supplements are foods that are a part of normal nutrition and represent concentrated sources of vitamins, minerals or other substances with a nutritional or physiological effect, individually or in combination. A large number of herbal remedies and herbal supplements on a plant basis exist on the market and are available to citizens without a prescription [11]. Interactions between drugs, dietary supplements, herbal remedies and herbal supplements based on plant and food can be both limit-

ing and life-threatening. Expressions of drug-nutrient interactions and food-drug interactions are often used as synonyms even among health professionals [11, 35]. Drug-nutrient interactions involve specific changes in the activity of the drug caused by nutrient/nutrients or changes in the drug-induced nutrient kinetics [11, 35]. Food-drug interactions are a broader concept that includes the effects of medication on nutritional status [36]. Today, and in the future, there will be more polypharmacy and self-medication, and the role of pharmacists in advising general population how to use drugs and dietary supplements in relation to nutrition is of enormous importance. Expert knowledge of drug-nutrient interaction and food-drug interaction pharmacist can be useful professional help to the doctor, and cooperation in the field of interactions must be wider and more efficient [37]. The existence of a growing market of dietary products and dietary supplements points to the need to get acquainted and trained pharmacists in the field of nutrivigilance as well as in pharmacovigilance. The pharmacist possesses knowledge in the field of phytotherapy, which has to be constantly improved in order to monitor new knowledge in the field of interaction of herbal remedies and on herbal supplements and nutrients [8]. The pharmacist's education on nutrition and food-drug interactions and in the field of nutrivigilance is a professional obligation, because knowledge of these areas significantly reduces the risk of health damage of drug users and dietary supplements users, increases the efficiency of pharmacological therapy and reduces the costs of treating adverse events [8, 11, 35–37].

Pharmacist in the Health Protection Caused by Environmental Pollution

In the modern society the role of pharmacist also includes the application of current knowledge about the direct impact of environmental pollutants on human health, especially the vulnerable population (pregnant women, toddlers, children, adolescents, elderly, patients with immunodeficiency and patients with chronic diseases). The harmful influence of pollutants both on humans and the environment as well as making pressure on legislators to limit their production, import and usage are the part of pharmacist role. The development of new food technological processes, consumer goods and cosmetics production, as well as the synthesis of new materials which are used in industry and everyday life has led to continuous low level impact of industrial chemicals on the environment and humans. Some of them are recognized as endocrine disrupting chemicals (EDCs), natural and synthetic substances which may interfere with the hormones by imitating or neutralizing their action. Among EDCs are genistein, daidzein, bisphenol A (BPA), phthalates, perfluorooctan acid (PFOA), perfluorooctan sulfonates (PFOS), polychlorinated biphenyls (PCB) and polybromated biphenyls (PBB). Based on novel literature data EDCs have negative effect on human health even in minimal concentrations and are connected not only with the disruption of the endocrine function, but also with noninfectious diseases such as obesity, diabetes mellitus type 2, cardiovascular diseases, metabolic syndrome, as well as the influence on the reproductive health of men and women, influence on pregnancy development, formation of the fetus and fetal development and physiological growth and development of children and adolescents [38, 39]. Pharmacist as one of the most available healthcare professionals has direct communication with the public, enjoys the public's trust, has the knowledge and opportunity to give professional information about the harmful effects of various anthropogenic pollutants especially endocrine disrupting chemicals [40]. Therefore, pharmacists have the important impact to interpret and to explain professional information to the complete population and specific vulnerable groups, and thus help in health protection [13].

Pharmacist and Good Manufacturing Practice

The European Union (EU) legislation in the pharmaceutical sector (EudraLex) is compiled in the publication of the European Commission "The rules governing medical products in the European Union" [41]. It consists of 10 volumes: volume 1 and 5 present basic legislation which is supported by series of guidelines (other 8 volumes). Volume 4 sets the guidelines for good manufacturing practice (GMP) for medical products for human and veterinary use. The purpose of GMP is to maintain high standards of quality management and to ensure that all medical products are safe, quality and efficient. Each country develops GMP guidelines following the international GMP standards. In the Republic of Serbia two current European Directives 2003/94/EC and 91/412/EEC that set the principals and guidelines of GMP are adopted in the national legislation and used as a basis for the best principles of quality management. Ministry of health and Medicines and medical devices agency of Serbia are responsible for creation of GMP guidelines as well as for the determination and control of laboratory tests compatibility with the GMP guidelines and that the end-point quality is reached [42]. Pharmacists need to respond to the challenges of 21st century by compiling regulation that incorporate novel scientific information and policy in order to successfully play an increasingly prominent role in the future healthcare.

Conclusion

Pharmacy has changed over the last few centuries from the manufacture and sale of vary of medical products to a range of important clinical services and public health roles. The pharmacists of the 21st century play remarkably broad roles including optimization of the drug delivery systems, personalized therapy, the prevention and control of chronic non-communicable diseases, advising general population how to use drugs and dietary supplements in relation to nutrition and the professional care about the possible negative effects of the environment pollution to the human health. All the mentioned roles of the modern pharmacist have to be covered by the current national and European Union legislation.

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VIRTUAL MICROSCOPY IN HISTOLOGY AND PATHOLOGY EDUCATION AT THE FACULTY OF MEDICINE, UNIVERSITY OF NOVI SAD

VIRTUELNA MIKROSKOPIJA U NASTAVI HISTOLOGIJE I PATOLOGIJE NA MEDICINSKOM FAKULTETU UNIVERZITETA U NOVOM SADU

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Summary

Both histology and pathology as a scientific fields and as an educational subjects have always relied on technology. In the 19th century a major breakthrough happened in certain areas related to histotechnology, so histology and pathology were rapidly developing. Technological revolution has lead to modernization of histology and pathology teaching, resulting in virtual microscopy. Advantage of virtual microscopy is an improved way of teaching and better cost-effectiveness. As a method of histology and pathology teaching, it was implemented at the Faculty of Medicine of the University of Novi Sad in 2016, in a specially equipped classroom at the Institute of Histology and Embryology. Virtual Local Area Network segregation is enabled in this classroom, which allows network traffic from different groups of users to be securely segregated, creating an independent environment for each student's computer. Students can simultaneously view audio-visual contents on their monitors, on projector screen and on 6 large 55-inch screens. Preexisting microscope glass slides with most representative tissue or organ sections and optimal staining quality were selected and scanned with *NanoZoomer S210 Digital slide scanner – Hamamatsu* that can rapidly scan glass slides and convert them to digital data. For viewing digital slides we use *NDP.view2* program. It allows moving, rotating, zooming and focusing of digital slides via the mouse or keyboard. Program enables morphometric measurements, colorful special pointers, annotations on slides and "bird's-eye-view".

Key words: Microscopy; Image Processing, Computer-Assisted; Education, Medical, Undergraduate; Students, Medical; Histology; Pathology; History of Medicine; Computer-Assisted Instruction

Sažetak

Histologija i patologija kao naučna polja i kao obrazovni predmeti, oduvek su se oslanjali na tehnologiju. U 19. veku su se desila značajna otkrića u okviru histološke tehnike, tako da su se histologija i patologija počele ubrzano razvijati. Tehnološka revolucija je dovela do modernizacije nastave u histologiji i patologiji, što je rezultiralo virtuelnom mikroskopijom. Prednost virtuelne mikroskopije je u poboljšanju načina predavanja i bolja isplativost. Kao metod nastave u histologiji i patologiji, implementirana je na Medicinskom fakultetu Novi Sad 2016. godine, u specijalno opremljenoj učionici Zavoda za histologiju i embriologiju. U ovoj učionici je omogućeno razdvajanje virtuelnih lokalnih mreža, koje opet omogućava da mrežni saobraćaj od različitih grupa korisnika bude sigurno odvojen, stvarajući nezavisno okruženje za svaki studentski računar. Studentima je na taj način omogućen prenos audiovizuelnih sadržaja preko pojedinačnih monitora, preko ekrana projektora i putem šest velikih ekrana od 55 inča. Stare staklene mikroskopske pločice sa najreprezentativnijim isečcima tkiva i organa i optimalnim kvalitetom bojenja su odabrane i skenirane pomoću *NanoZoomer S210 Digital slide scanner – Hamamatsu* koji može brzo da skenira staklene pločice i pretvori ih u digitalne podatke. Za pregled digitalnih slajdova koristimo *NDP.view2* program. On omogućava pokretanje, rotiranje, uveličavanje i fokusiranje digitalnih slajdova pomoću miša ili tastature. Program omogućava morfometrijska merenja, šarene specijalne pokazivače, primedbe na slajdovima i pogled iz ptičje perspektive.

Ključne reči: mikroskopija; kompjuterska obrada slike; osnovne studije medicine; histologija; patologija; istorija medicine; edukacija uz primenu računara

Histology before Subject "Histology"

In medical and biological science and education, histology is a relatively new discipline, although it has been studied much earlier than it was named. Centuries ago, from the time of Antonie van Leeuwenhoek (1632–1723), microscopic structure of tissues was recognized and analyzed. Perhaps one of the most important discoveries in this field was a discovery of pulmonary capillary network of frog, by Marcello

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Abbreviations
LM – light microscope
VM – virtual microscopy

Malpighi (1628-1694), who was named the "father of histology". Next man designated as the "father of histology" was Marie F.X. Bichat, who described 21 types of tissues in 1801, but without using microscope. The term "histology" was finally introduced in 1819, by Carl Mayer, professor of anatomy in Bonn (*Ueber Histologie und eine neue Eintheilung der Gewebe des menschlichen Körpers*) [1, 2].

19th Century Scientific Revolution in Histology

Histology as a scientific field and as an educational subject has always relied on technology. It is believed that first microscope was constructed in 1590, with magnification not greater than 10x, but it was Galileo Galilei in 1609 who explained and implemented the laws of physics in the construction of microscopes [2]. His work was continued through centuries, and the introduction of reliable, high-quality light microscopes more than 150 years ago enabled analysis of tissues and cell structures at an increasingly smaller scale. At the same time, in the 19th century, modernized histological techniques enabled microscopic observations to influence medicine development, practice and education [3]. Histological techniques, particularly paraffin wax sections and staining by *haematoxylin (Waldeyer W, 1864*), have become developed enough to provide understanding of tissue structure, and pose it as a scientific base of physiology and pathology. All of this has lead to great discoveries in the beginning and middle part of 19th century: cellular theory (*Mathias Schleiden and Theodor Schwann*), cellular pathology (*Rudolf Virchow*) and microorganisms as a cause of diseases (*Louis Pasteur*), which all stand today as foundations of modern medicine [1].

Histology in 19th and 20th Century Medical Education

Histology became part of medical education curriculums at the end of 19th and beginning of 20th century. The first textbook with histology material was published by Jacob Henle in 1841 (*Allgemeine Anatomie*) and next by Arthur Hassall in 1846 (*Microscopic Anatomy of the Human Body in Health and Disease*), and the first textbook containing only histological topics and knowledge was published by Rudolf Albert von Koelliker in 1852 (*Handbuch der Gewebelehre des Menschen*). At this point of time, histological technique was underdeveloped, and their examinations were very simple, on native tissue in unstained slides [1, 2].

Changes in histology course materials at the beginning of 20th century have reflected improvements in histological techniques and slide preparation as well as developments in light microscopes and associated photomicroscopy [4]. At this time, histology textbooks become modern, representing histology as we known today [2]. One of the most famous European textbooks was written by Ladislaus Szimonowitz (*Lehrbuch der Histologie und mikroskopischen Anatomie*, 1901), from Lemberg (today Lviv, Ukraine), and it was translated to many world languages. One of the world's most respected textbooks in histology was written by Russian author Alexander Maximow in 1915. It was translated into English language by William Bloom (*A textbook of histology*, 1930, Philadelphia: W. B. Saunders), and it had 12 editions [1].

Changes in course content during the 20th century initially emphasized new knowledge of structure as observed at the light and electron microscope levels. Along with light microscopy, transmission and scanning electron photomicrographs were used in teaching histology during the second half of the 20th century. Academic teachers subsequently incorporated more histophysiology and histopathology into their courses to emphasize newly acquired information on the function and clinical relevance of the cells and tissues studied [4]**.** Until today, a series of the most respectable histological textbooks were published in Springer by professor Radivoj Krstić, histologist from Novi Sad and Lausanne, translated into German, English, Frensh, Russian, Italian, Japanese, and Spanish language.

Beginning of Histological Education in Serbia

Histological experimental work and teaching in Serbia started at the Belgrade Higher School (latter developed into University of Belgrade) at the end of 19th century. The first microscopes were transferred from the Palace of King Milan into Belgrade Higher School, so that Živojin Đorđević (1872-1957), the professor of zoology in this school, could start with histological technique and histological education from 1899. Eduard Mihel (1864-1915), the first pathologist in Serbia, started with histological technique in prosecture at the General State Hospital in Belgrade, in 1896. He was interested also in truly histological questions, like neuroglia, modern topic at that time, and published paper about them in Serbian Archive for General Medicine.

Institute of Histology at the Faculty of Medicine in Belgrade, established in 1921 by Aleksandar Kostić, was the foundation for the education of histology in Serbia. Under the influence of professor Pol Bouin from histological schools in Nancy and Strasbourg, professor Kostić introduced modern methods in research and education at the Institute of Histology. From 1921, he published series of histology manuals and textbooks, with many original photographs of histological slides [5]. Finally, all Serbian histologists active today, are disciples of professor Kostić, as they all have studied histology from the last edition of his great textbook of histology in 1980s. One of his apprentices, academician

Figure 1. Classroom of professor Milin
Slika 1. *Učionica profesora Milina*

Radivoj Milin was the founder of Institutes of Histology at the Faculties of Medicine in Sarajevo (1950) and in Novi Sad (1960). At the time, classrooms for histology practical lessons had monocular microscopes **(Figure 1)**.

Virtual and Light Microscopy in 21st Century Medical Education - Advantages and Disadvantages

Long before the availability of colour-printed textbooks and the advent of personal computers and portable electronic devices, the best method by which students learned about histological, biological and pathological entities was by viewing specimens through light microscopes (LM) [6]. Over the last decade new technological advances have resulted in significant changes how we teach histology and pathology, and one of them is the abandonment of LM in favor of digital histological images, referred to as "virtual microscopy" (VM). Students can access these images through local networks or the Internet [3]. More and more institutions of higher education offer histology and pathology courses that partially or entirely rely on virtual microscopy, as a main teaching tool. In 2009, about 50% of pathology courses in the United States already have or expect to implement virtual microscopy [7].

So why is the use of LM no longer "cool" for teaching histology and pathology? Some of the usually mentioned disadvantages of LM are [3, 6]:

– constant financial drain due to maintenance of a large number of student microscopes and sizeable collections of glass slides;

– great disparities between the learning resources that are available to individual students due to variable quality of histological preparations and difficult acquisition and replacement of many tissues, especially of human origin;

– students lack experience in using LM before undergraduate medical school, so their LM usage skills and etiquette are poor, and they need time to master it, but the time dedicated to basic medical-science practical sessions in integrated training systems is insufficient;

– low accessibility to microscopes and slides, due to their limited number or spatial overlapping with other courses (for example, when same classrooms are used by several educational subjects).

It is not only technical and financial aspect that should be taken into consideration, but also a fact that we are teaching a traditional subjects to today's "computer-savvy" generation of students who explore and utilize all possible virtual and electronic resources.

Positive aspects of traditional LM in teaching process are developing skills required to manipulating a LM and to appreciate the variability of the biological material they have at their disposal.

Advantages of VM are numerous, based on pedagogy, efficacy and cost [3, 6, 7, 8–11]:

– provides students with a viewing experience that is very comparable to real histological glass slides;

– every student has equivalent access to the highest quality slide material;

– adequate software allows each student to select specific regions of interest on the slide, to zoom in and out and to move to other areas at their free choice;

– at high magnifications it is easy for the student to maintain orientation with respect to the entire section;

– image is always in focus, with optimized contrast and adjustable virtual illumination;

– use of labels enhances learning process, and many other.

– students and professors adapt very quickly to the use of the virtual microscope;

– single-use microscope laboratory can be converted into a multi-use computer laboratory;

– after an initial investment in the scanning stage, software and servers, the financial and administrative advantages allow enormous economic savings;

– in the long-term with regard to equipment, technical staff and laboratory facilities.

Virtual microscopy has its own drawbacks: it delivers only a single plane of focus, thus lacking the three-dimensionality, which students can obtain using the fine focus knob of a regular microscope. In addition, VM relies heavily on a stable technological infrastructure that must accommodate multiple users accessing the same slide simultaneously and potential server outages [3, 6]**.** There were also some drawbacks recognized during the hours of histology practical, like a tendency of some students to passively follow the demonstration of digital slides. This should be minimized by the proper interactive engagement of students in the practical histology session [12].

The classic LM enthusiast argues against the use of simulators as VM, claiming that they can fundamentally alter the essence of medical education, and

in contrast, technology aficionados may be infatuated with new inventions and be too quick to adopt new technologies without validating them. This can cripple students' abilities to adapt and deal with real-life situations [3]. We believe that adequate monitoring of students' impressions and performance can prevent such an unfavorable prognosis.

Students' Impressions and Achievements

As new technology in teaching process is being implemented, students' perception must be taken into consideration. Study conducted by Holaday et al. at the University of Michigan Medical School (class of 2014), looked at the usage of various electronic and traditional histology-learning resources, and revealed two important tendencies. In general, most students preferred to study histology at their own time and scheduled resources, such as lectures and lab sessions, suffered a decreasing attendance as the academic year progressed. This finding is in accordance of our own observations at the Department of Histology and Embryology. The second major finding showed a strong and growing preference to use a variety of electronic resources, not only VM [13]. In the study of Foad et al. students appreciated the ease of using VM vs. LM and found the former more interactive and that continuous feedback from tutors minimizes boredom and knowledge gaps. Students gained skills for the use of the VM materials swiftly. As the result, the duration of the sessions can be reduced, or students can spend extra time in validating the skills attained. In contrast, the LM group's skills had a steep learning curve, and often valuable time during the sessions was dedicated to adjusting the microscopes' fields, power and focus [6]. Our own observations during VM teaching and students' comments correlate to the results of this study.

Students analyzed by Ostrin et al. declared to have much higher motivation when using VM in histology classes [9]. Questioners used by Anyanwu et al. showed that 78% of students have some kind of problems using LM, 68% of students think they understand histology better when using VM, while 73% believe that they have better chances in passing the histology practical exam if conducted by VM [10].

With the introduction of new technologies such as virtual microscopy the question arises whether students' performance suffers? Foad et al. compared success of both methods by written and practical exams. Students in the VM group performed better than those in the LM group in both practical and written exams, as reflected by their more-uniform performance and less-scattered grades. The virtual microscopy group had the advantage of optional online off-campus access to study materials, which they spent an average of 2.5 h reviewing [6]. Study by Anyanwu et al. showed same results in students' impressions and performance, with the information that the costs of conducting examination using VM were significantly reduced [10].

Collier et al. surveyed teaching assistants for their acceptance of VM use as a teaching tool for undergraduate students in histology. They advocated the use of VM besides providing the students with access to LM. Some researchers affirmed that VM "can effectively replace the traditional methods of learning pathology" [14].

Integration of Virtual Microscopy in Teaching Process at the Faculty of Medicine, University of Novi Sad

Up until 2013 at the Medical Faculty in Novi Sad, classical light microscopes were used to perform exercises in the histology subject. The large number of students distributed by groups, the lack of sufficient histological slides, and frequent microscopic failures were the main reasons for changing the form of teaching. During the next two school years (2013/2014 and 2014/2015), all student microscopes were replaced by a central microscope that, using a microscope camera connected to the computer, enabled the "live" image to be projected onto the projection screen. A similar form of teaching is present today at the Medical Faculty in Nancy, France. This type of teaching enabled the trainer to achieve greater interaction with students. However, this mode of teaching also had some technical shortcomings. One of the greatest shortcomings is that students that are the farthest from the projection screen could not see clearly histological details on the screen due to poor resolution.

During 2015, thanks to Ivan Milenković MD, PhD, an assistant professor of the Institute of Neurology Medical University of Vienna, we enabled to scan the entire microscopic collection in high resolution for both Departments, Histology and Embryology and Pathology, respectively. That same year, thanks to the funds of the Provincial Secretariat for Higher Education and Scientific Research, intended for raising the quality of teaching, 25 computers were purchased for analysis of scanned slides. This type of teaching was used during the next two years (2015/2016 and 2016/2017). Faculty of Medicine University of Novi Sad, led by dean professor Snežana Brkić MD, PhD recognized the importance of modernization in histology and pathology teaching methods, and as part of the reconstruction of the Institute of Histology and Embryology, in June 2017 the first modern classroom for performing VM teaching was formed.

Making a classroom adequate and fully equipped for implementation of virtual microscopy required many infrastructural investments: the purchase of specially made tables with 2 places for students with electricity and internet connections; 38 computers and monitors; 6 large screens as well as video projector. VLAN (Virtual Local Area Network) segregation is enabled in this classroom, which allows network traffic from different groups of users to be securely segregated, creating an independent envi-

ronment for each group or customer (student's monitor/computer). Users of this multifunctional classroom can simultaneously view audio-visual contents on their monitors, on projector screen and on 6 large 55-inch screens **(Figure 2)**.

Such equipped classroom is optimal space for theoretical or practical lessons and practical exams in histology and pathology. Moreover, due to its multifunctionality, it is suitable for all kinds of presentation. In honor of the reestablishment of the Institute of Histology and Embryology at the Faculty of Medicine University of Novi Sad, a symposium "News in Histology and Embryology" was held in June 2017, and it went perfectly. This has established another possible usage of such multifunctional classroom.

For making virtual microscopy slides we used preexisting microscope glass slides of different tissues and organs. These slides were used as teaching collection for students, while light microscopy was used as a method of teaching. Slides with the most representative tissue or organ sections and optimal staining quality were selected and scanned with *NanoZoomer S210 Digital slide scanner - Hamamatsu* which is whole slide scanner that can rapidly scan glass slides and convert them to digital data. This scanner can automatically scan up to 210 slides. Dimensions of compatible glass slide are 26 x 76 mm with thickness from 0.9 to 1.2 mm. Scanning resolution on this scanner for 20x mode is 0.46 µm/pixel (with scanning speed of approximately 60s) and 0.23 µm/pixel for 40x mode (with scanning speed of approximately 150s) [15].

For viewing digital slides we use NDP.view2 program. This software is used for displaying digital slide files of histopathological samples (NDPi files, NDPis files, VMS files and VU files) that have been created on the *Hamamatsu NanoZoomer system*. It allows more responsive and smooth panning (moving and rotating), zooming and focusing functionality via the mouse, keyboard or other devices of various digital slides. Within the program, it is also possible to perform morphometric measurements, set of colorful special pointers and annotations on slides, as well as the ability to store images

Figure 2. Nowdays virtual microscopy classroom
Slika 2. *Današnji izgled virtuelne mikroskopske učionice*

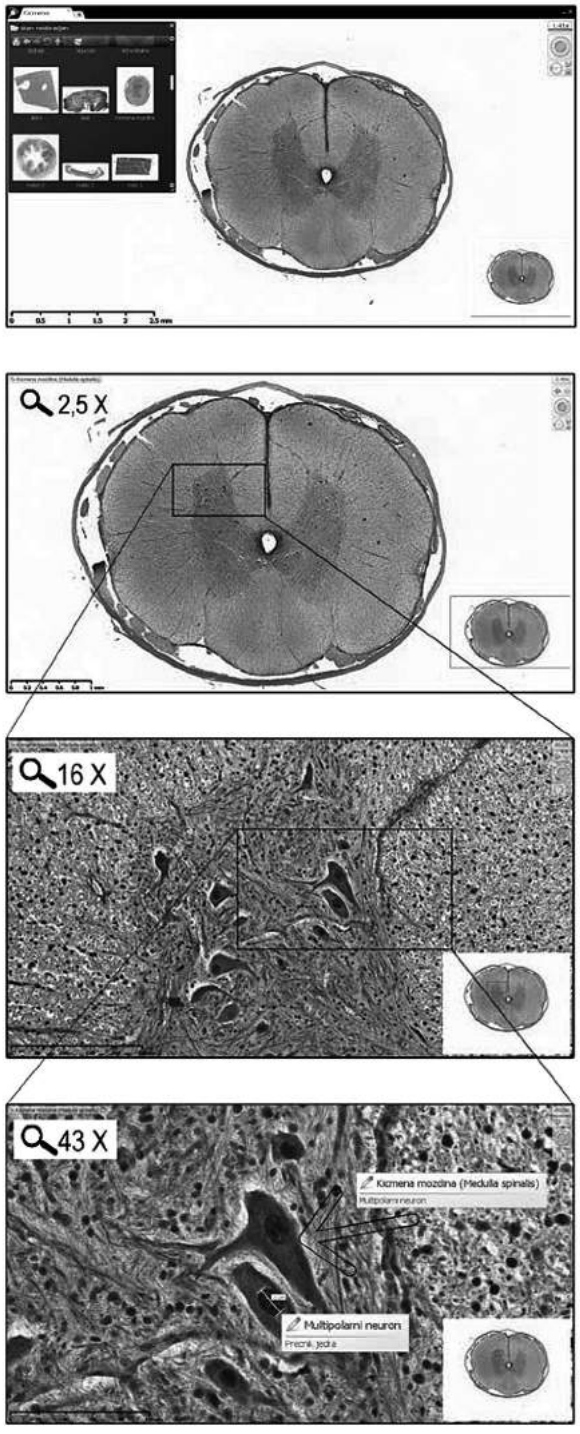

Figure 3. Screenshots of NDP view 2 software in different magnifications with labels and annotations
Slika 3. *Prikaz NDP-view 2 softvera pri različitim uveličanjima sa oznakama i pribeleškama*

in different formats (.jpg, .bmp, .tiff). Also NDP. view2 offers a "birds-eye-view" specific only for this software **(Figure 3)** [16]. Today, a collection of scanned digital slides of the Department of Histology and Embryology and the Department of Pathology used in practical exercises counts 200 slides.

Our Future Goals

After successful technical implementation of VM in the Histology and Embryology and Pathology courses, other goals must be achieved. As stated above, careful monitoring of students' impressions on both good and bad sides of VM must be observed. Analysis of exam performance, questionnaires on students' satisfaction and assessment of knowledge retention are also planned.

Since our institution provides integrated studies in Medicine and Dentistry organized in English language, many students are not citizens of Serbia so e-learning and broad on-line availability of virtual slides are of exceptional significance. And finally, since the pace of change in education and technology is not slowing down, development of novel interactive teaching tools and approaches that are highly valued by today`s students will be our point of interest.

Conclusion

Despite potential shortcomings, when compared to the traditional, microscope based approach of teaching histology and pathology, virtual microscopy is at least comparable, if not more effective. Most students and academic teachers appear to have enthusiastically embraced this change of teaching modus with no indication that learning success has been compromised. When carefully used in the context of a coherent didactic program the advantages of adopting virtual microscopy and other electronic educational media clearly outweigh their limitations.

However, the knowledge of how to operate a regular light microscope is still a useful skill. This not only applies to the research environment, but also to some clinical settings, especially pathology, microbiology, embryology and others.

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EYE CARE IN MECHANICALLY VENTILATED CRITICALLY ILL ADULTS –NURSING PRACTICE ANALYSIS

NEGA OKA KRITIČNO OBOLELIH PACIJENATA NA MEHANIČKOJ VENTILACIJI – ANALIZA SESTRINSKIH INTERVENCIJA

Dragana MILUTINOVIĆ[1], Dušanka CVIJANOVIĆ[2], Zlatko ĆIRIĆ[3], Gordana JOVANOVIĆ[4] and Ana ANDRIJEVIĆ[3]

Summary

Introduction. Eye care is among basic nursing interventions in critically ill patients, but often neglected due to an ongoing focus on life threatening conditions in this group of patients. At the time being, there are no national guidelines for eye care in critically ill patients in Serbia. Aim of the study was to evident nurses' knowledge, attitudes and everyday clinical practice toward eye care in mechanically ventilated critically ill patients. **Material and Methods.** The study was prospective, observative and cross sectional. Nurses working in intensive care units were interviewed. Study instrument was self administered questionnaire - "*Eye care clinical competence in ICU inventory - ECC"*. This questionnaire showed good reliability with Cronbah Alfa 0.83. Descriptive and inferental statistical analysis was conducted in data analysis,with statistical significance of $p<0.05$. **Results.** Total average score of knowledge, attitude and everyday practice test point out that further improvement in the quality of eye care is needed in mechanically ventilated patients. There is a strong positive correlation between attitudes and practices in eye care - the more positive attitudes lead to more quality practices. **Conclusion.** According to our study results, nurses generally think that eye care in mechanically ventilated patient is important, but a general awareness about practices of care and prevention of iatrogenic eye conditions could be improved.

Key words: Eye Injuries; Corneal Injuries; Intensive Care Units; Nursing Staff, Hospital; Critical Illness; Surveys and Questionnaires; Health Knowledge, Attitudes, Practice; Respiration, Artificial

Sažetak

Uvod. Nega oka je jedna od bazičnih sestrinskih procedura koja se sprovodi kod pacijenata u jedinicama intenzivne terapije, ali zbog fokusiranosti na rešavanje životno ugrožavajućih stanja ova procedura je često zanemarena. Situaciju otežava i nepostojanje jedinstvenog vodiča dobre prakse za negu oka. Cilj ove studije bio je procena znanja, stavova i prakse medicinskih sestara zaposlenih u jedinici intenzivne terapije o nezi oka pacijenata na mehaničkoj ventilaciji. **Materijal i metode.** Ispitivanje je sprovedeno kao opservativna, analitička studija preseka, anketiranjem medicinskih sestara (N = 95) zaposlenih u jedinicama intenzivne terapije. Kao instrument istraživanja korišćen je *Upitnik kliničke kompetencije za negu oka u jedinicama intenzivne terapije*. Upitnik je pokazao dobru pouzdanost, Kronbah (*Cronbah*) alfa za ceo upitnik iznosio je (0,83). Za statističku obradu podataka primenjene su metode deskriptivne i inferencijalne statistike, a statistička značajnost određivana je na nivou $p < 0,05$. **Rezultati.** Prosečni ukupni skorovi dobijeni na testu znanja stavova i prakse ukazuju na potrebu unapređenja kvaliteta usluga koje se pružaju pacijentima na mehaničkoj ventilaciji. Između stavova i prakse prema nezi oka pacijenta na mehaničkoj ventilaciji dobijena je jaka pozitivna korelacija, odnosno pozitivniji stav prati bolja praksa. **Zaključak.** Prema rezultatima naše studije, medicinske sestre generalno smatraju negu oka važnom, ali nisu dovoljno svesne mera predostrožnosti i postupaka kojih se treba pridržavati radi prevencije i tretmana oštećenja površine oka kod kritično obolelih i povređenih pacijenata.

Ključne reči: povrede oka; povrede rožnjače; jedinice intenzivne nege; bolničko osoblje; kritično oboleli; ankete i upitnici; znanje o zdravlju, stavovi, praksa; mehanička ventilacija

Introduction

Eye care is an important component of a daily nursing care plan. It consists of regular eye assessment, and prevention of iatrogenic ophthalmological complications (cleaning of the eye with normal saline or sterile water, closure of the eyelide using either ocular lubricant, or creation of a moisture chamber using polyethylene wrap). Eye care should be provided to all hospitalized patients, especially to patients in intensive care units, in whom regular protective mechanisms of the eye are often impared [1].

Healthy persons have physiological mechanisms for eye protection. By blinking and eye closure,

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Abbreviations
ECC – Eye care clinical competence

tears cover eye surface and prevent mechanical injuries and microorganism colonization. At the same time, blinking prevents tears evaporation and desiccation of the eye. Physiological tear production is important for corneal epithelium, acting as a lubricant, maintaining the moisture of the eye, nurturing epithelial cells of the cornea and clearing mechanically small foreign particles from the eye [2, 3].

Majority of critically ill patients in intensive care units, having altered state of consciousness (due to sedation or brain conditions) lose protective eye mechanisms. It can lead to eye dryness, infection, ulcerations, even perforation and iatrogenic mechanical corneal injuries, with the end result of visual impairment and decreased quality of life [4].

Everyday care for critically ill patients (sedated and mechanically ventilated) with certain procedures and drugs, can lead to diminishing this physiological protective eye mechanisms too. Sedatives and neuromuscular blocking drugs inhibit eye muscles and lead to lagophtalmus-noncomplete eyelid closure, which can lead to iatrogenic eye conditions. Different states as circulatory volume overload, high blood vessel permeability and inadequate endotracheal tube fixation can lead to a reduction of venous drainage from the eye, edema of the eye and lagophtalmus as a consequence. Lagophtalmus can lead to infection due to exposure of the eye to numerous pathogens in the intensive care environment [5–7]. Mechanically ventilated patients with positive end-expiratory pressure (PEEP) more than 5 mmH_2O can develop a condition called "ventilatory eye" and iatrogenic corneal injury, due to decreased venous drainage, conjuctival edema and chemosis [8]. Infection risk is higher in patients who require frequent enotracheal suctions, especially if there is an inappropriate technique [9].

According to Güler et al. the incidence of iatrogenic eye conditions (conjunctivitis, lagophtalmus, corneal abrasions, keratopathy, dry eye, microbial keratitis, endophtalmitis) in patients in intensive care units range from 3.6% to 89.3% [10]. Lagophtalmus prevalence in the study conducted by Sivasankar et al. in sedated patients in intensive care units, range from 20% to 75% [11]. The incidence of corneal abrasions in mechanically ventilated patients is from 3 to 60%, and occuring between second and seventh day of hospitalisation [2, 12].

Taking into consideration high prevalence and profound impact of iatrogenic eye conditions on quality of life, eye care in critically ill is fundamentally important practice. Focus on treatment of critically ill patients is on life treathening conditions, and sometimes eye care is neglected [2]. All studies are uniform in stating that there is not enough knowledge about the importance of nursing eye care procedures due to lack of proper guidelines. Positive attitudes and practices of nursing personnel are prerequisite for eye care improvement in intensive care units [10].

The aim of the study was to examine knowledge, attitudes and nursing practices toward eye care in critically ill patients on mechanical ventilation.

Material and Methods

The research was carried out in the form of a descriptive, analytical and cross-sectional study, by interviewing nurses (N = 257) who worked in three intensive care units: Emergency Center (n = 33, 34.7%), Clinic for Anesthesia and Intensive Care, Clinical Centre of Vojvodina (n = 22, 23.2%) and Institute for pulmonary diseases of Vojvodina (n = 40, 42.1%). The study was conducted from December 2016 to January 2017.

The instruments used in this study were two self administered questionnaires: "*Eye care clinical competence in ICU inventory - ECC"*, and general questionnaire, for gathering sociodemographic data (gender, working experience and educational status).

Eye care clinical competence in intensive care units inventory is created by Ebadi et. al. in 2015. It comprises of 35 items gathered in three parts. The first part is comrpised of 18 multiple choice questions for the assessment of knowledge about eye care and iatrogenic eye conditions (causes, treatment and nursing practices) in critically ill patients. The second part is comprised of seven items assessing attitudes towards the importance of eye care nursing procedures. The third part is comprised of 10 items assessing current practices in eye care in mechanically ventilated patients and their frequency.

Knowledge domain is scored with 1 (correct answer) or 0 (incorrect answer). Five points Lickert scale is used for attitudes domain: 1 = not important to 5 = very important. Five points Lickert scale is used for nursing practice domain, 1 = never to 5 = always. In each domain higher score means better knowledge, more positive attitude and better nursing practice.

Psychometric testing of care clinical competence (ECC) test conducted by the authors showed good internal consistency with Cronbach' alfa for attitudes domain of 0.76, and for nursing practice domain of 0.85. Internal consistency of knowledge domain is tested by Kuder-Richardson 20 test and value of 0.83, showing its good reliability [13]. In our study ECC as a whole showed good reliability. Cronbach' alfa was (0.83) for the entire questionnaire, for knowledge domain (0.78), attitudes domain (0.76) and nursing practice domain (0.86).

Descriptive statistics were used in determining mean, standard deviation, minimum and maximum values, and 95% confidence interval and absolute frequencies, according to the variable type. The normal distribution is assessed by the Kolmogorov Smirnov test ($p>0.05$). Pearson correlation coefficient was used to determine a relationship between the parametric variables. Comparison of differences between means from two different groups was done by t-test. One-way ANOVA variance analysis was used to compare the means of multiple groups, with post-hoc Tukey's

range test. The data were analyzed by IBM SPSS 23 Statistics software with statistical significance $p < 0.05$.

The study was approved by the Ethical committee of the Clinical Centre of Vojvodina, document (00-15/23) and the Ethical committee of the Institute for pulmonary disease of Vojvodina.

Results

From the total number of nurses partcipating in to study N = 95, there were 69 (72.6%) females, and 26 (27.4%) males nurses. Majority of nurses had secondary school education 85 (89.5%), while 10 nurses (10.5%) hold college or bachelor's degree **(Table 1)**. Average working experience was 8.5±7.6 (SD) years, ranging from three months to thirty two years.

Average scores on the knowledge test, considering causes, treatment, and eye care was 6.3±2.5 (SD) from 18 points in total. Two thirds of nurses knew causes for the blink reflex impairment, and the same number of nurses n = 74 (77.9%) did not know the causes for corneal abrasions. Less than half of study nurses 44 (46.3%) knew the proper eye cleaning technique and 10 (10.5%) nurses did not know what the purpose of eye care in the Intensive Care Unit is **(Table 2)**.

Analysis of the total average scores in the knowledge domain on ECC test by Student t – test for independent samples showed statistically significant difference, according to education level and gender in the study sample **(Table 3)**.

On the knowledge domain difference between total and avearge score on ECC test were statistically significant ($F = (2, 92) = 6.656$, $p = 0.002$), according to nurses'working place, while there was no statistically significant difference according to working experience.

On the attitude domain, total average score on ECC test was 25.6±3.6 (SD), ranging from 16.1 to 30.7, implicating mild positive attitude. Although, 30 (31.5%) nurses did not consider eye care protocols to have any impact on corneal abrasion incidence, and 29 (30.6%) nurses considered eye care to have low priority in critically ill patients on mechanical ventilation. The difference in attitudes toward nursing interventions in preventing iatrogenic eye conditions in patients on mechanical ventilation were apparent according to gender ($t = 0.046$; $df = 93$, $p < 0.05$) and working experience ($F = (2.92) = 6.176$, $p = 0.003$). Male gender and working experience more than 10 years had more positive impact on nurses' attitudes.

On the nurses' practice domain, total average score on ECC test was 32.8±7.8 (SD), ranging from 17.2 to 45.5, showing that there is a need for improvement in a quality of care for patients on mechanical ventilantion. Rarely or never, nurses n=26 (27.3%) use normal saline or sterile water for eye cleaning every two hours, 47(49.5%) do not use lubricant, 41 (43.1%) eyedrops and 32 (33.7%) eye ointment **(Table 4)**.

Nursing practices are not impacted by educational level of nurses, working experience, gender or health facility they are working in.

There was no statistically important correlation in knowledge between prevention, eye care treatment, attitudes and nursing practices in mechanically ventilated patients.

There is a strong positive correlation $r = 0.529$, $p = 0.00$ between attitudes and nursing practices **(Table 5)**. More positive attitude was accompanied by better nursing practices.

Discussion

There are insufficient number of studies on eye care in intensive care units. It is a global problem in developed and developing countries like Serbia. In 2015 healthcare institutions in Serbia passed the accreditation procedure for providing health care services, but insight into the existing available procedures noted the lack of a written guidelines for eye care. This fact confirms that nurses are still not aware of the importance of eye care in critically ill patients. Therefore, this study was conducted in two major tertiary University hospitals in Vojvodina in order to assess knowledge, attitudes and current

Table 1. Sociodemographic characteristics of nurses
Tabela 1. *Sociodemografske karakteristike medicinskih sestara*

		n	%
Gender *Pol*	Female/*Ženski*	69	72.6
	Male/*Muški*	26	27.4
Total/*Ukupno*		95	100
Educational level *Stepen obrazovanja*	Secondary school/*Srednja škola*	85	89.5
	College and bachelor's or master's degree/*Viša i visoka škola*	10	10.5
Total/*Ukupno*		95	100.0
Working experience *Radno iskustvo*	< 3	32	33.7
	3.1 – 10	34	35.8
	> 10.1	29	30.5
Total/*Ukupno*		95	100

n = Absolute frequency/*Apsolutna učestalost*; % = Relative frequency/*Relativna učestalost*

Table 2. Correct and wrong answers distribution in knowledge domain on ECCI test
Tabela 2. *Distribucija tačnih i netačnih odgovora na pitanja iz domena znanja na Upitniku kliničke kompetencije za negu oka*

Questions for assessment of knowledge about eye care and iatrogenic eye conditions (causes, treatment and nursing practices)/*Pitanja za ocenjivanje znanja o nezi očiju i jatrogenim povredama oka (uzroci, tretman i sestrinske intervencije)*	True *Tačno* n (%)	False *Netačno* n (%)	I don't know *Ne znam* n (%)
Which of the following factors disturbs blink reflex? *Koji od sledećih faktora ometa refleks treperenja?*	72 (75.8)	16 (16.8)	7 (7.4)
Which of the following choices is a potential risk factor for eye disorders? *Koja od ponuđenih mogućnosti predstavlja potencijalni rizik za oštećenja površine oka?*	12 (12.6)	74 (77.9)	9 (9.5)
What is the most important criterion in assessing eye disorders in ICU? *Koji je najznačajniji kriterijum za utvrđivanje oštećenja površine oka u JIN?*	54 (56.8)	35 (36.8)	6 (6.4)
Which factors aggravate Chemosis?/*Koji faktori pogoršavaju hemozu?*	15 (15.8)	66 (69.5)	14 (14.7)
The best time for beginning and administrating eye care for patients hospitalized in ICU is/*Najbolje vreme da se započne nega očiju kod pacijenata hospitalizovanih u JIN je*	66 (69.5)	25 (26.3)	4 (4.2%)
How often should a patient be assessed regarding the protective mechanisms of the eye (ability to blink, etc.)?/*Koliko često treba proveravati zaštitne mehanizme oka kod pacijenta (sposobnost treptanja, itd.)?*	64 (67.4)	27 (28.4)	4 (4.2%)
How should endotracheal suctioning be performed to prevent eye complications? *Kako treba da se izvede endotrahealna sukcija kako bi se sprečile komplikacije očiju?*	17 (17.9)	71 (74.7)	7 (7.4)
What is the proper way for cleansing patient's eyes? *Koji je ispravan način za čišćenje očiju pacijenta?*	44 (46.3)	46 (48.4)	5 (5.3)
What is the appropriate size for eye pads and covers? *Koja je odgovarajuća veličina tufera i maske za oči?*	35 (36.8)	52 (54.8)	8 (8.4)
How should eye care be provided for a patient who can blink and close his eyes completely?/*Kako treba pružiti negu za oči pacijentu koji može potpuno da trepne i zatvori oči?*	20 (21.1)	65 (68.4)	10 (10.5)
What is the best eye care for a patient who cannot close his eyes and his sclera is exposed?/*Koji je najbolji tretman za oči pacijenta koji ne može da zatvori oči i beonjače su izložene?*	23 (24.2)	69 (72.6)	3 (3.2)
How should eye care be provided for a patient who is unable to blink and hence, his sclera and pupil are exposed?/*Kako treba obezbediti negu očiju za pacijenta koji nije u stanju da trepne, a beonjača i zenice su izloženi?*	56 (58.9)	33 (34.8)	6 (6.3)
How should eye care be given to a patient who receives mechanical ventilation and sedative agents?/*Kako uraditi negu očiju pacijentu koji je na mehaničkoj ventilaciji i sediran?*	49 (51.5)	43 (45.3)	3 (3.2)
The key objective of eye care is:/*Glavni cilj nege oka je:*	25 (26.3)	60 (63.2)	10 (10.5)
The best eye care plan is:/*Najbolji plan za negu očiju je:*	20 (21.1)	72 (75.8)	3 (3.2)
Which of the following methods is the most effective for preventing corneal abrasion?/ *Koji od sledećih metoda je najefikasniji za sprečavanje abrazije rožnjače?*	13 (13.7)	69 (72.6)	13 (13.7)
Eye cleansing by normal saline:/*Čišćenje očiju normalnim fiziološkim rastvorom:*	14 (14.7)	65 (68.4)	16 (16.8)
What is the right direction for applying adhesive tape on eyelids for closing the eyes? *Koji je pravi smer za nanošenje trake na kapke da bi oči bile zatvorene?*	13 (13.7)	78 (82.1)	4 (4.2%)

n = Absolute frequency/A*bsolutna učestalost*; % = Relative frequency/*Relativna učestalost, ICU - Intensive care unit/JIN - Jedinica intenzivne nege*

nursing practices in eye care in mechanically ventilated critically ill patients.

In our study majority of nurses (three quarters) acknowledged sedatives and neuromuscular blocking drugs in mechanically ventilated patients having a negative impact on physiological eye protection mechanisms. Very similar results are found in current literature [10, 14].

Interestingly, a half of the nurses interviewed in our study did not recognize endotracheal suction as a confounding factor for eye infections. Even half of nurses stated that endotracheal suction does not have any impact on eye infections in mechanically ventilated patients. In IC Manual best practice for intensive care: Eye care for critically ill adults, authors suggest best practices for endotracheal suction in order to prevent iatrogenic infections in intensive care units [9]. During this procedure, the nurse should stand on the lateral side of the patient with mandatory preventive covering of the patient's eyes. Only 17% of nurses recognized the correct answer. Correct fixation (securement) of endotraacheal tube is of outmost importance, because very tight fixation can lead to conjuctival edema. Asessment of

Table 3. Total average score in the knowledge domain on ECC test: differencies according to sociodemographic charcteristic of nurses
Tabela 3. *Ukupni prosečni skor na Upitniku kliničke kompetencije za negu oka u domenu znanja: razlike prema socio-demografskim karakteristikama medicinskih sestara*

	Mean ± SD *Prosek±SD*	t-test	95% CI	p	Cohen's d indicator *Kohenov d indikator*
Educational level/*Stručna sprema*					
Secondary school/*Srednja škola*	6.2±2.6	-1.721	-3.111 do -0.222	0.05	0.7 (medium effect *srednji uticaj*)
College and bachelor's or master's degree *Viša, visoka ili master*	7.6±2.0				
Gender/*Pol*					
Male/*Muški*	7.3±2.6	-2.433	-2.514 do -0.254	0.02	0.6 (medium effect *srednji uticaj*)
Female/*Ženski*	5.9±2.4				

endotracheal tube fixation is conducted only by 50% of nurses in our study [15].

In the current literature data show that conjuctival edema is present in 56.3% of patients in intensive care units, purulent secretion in 36.3%, retinal damage in 15% and ceratitis in 10% of patients [16]. According to IC Manual best practice of intensive care: Eye care for critically ill adults, this iatrogenic eye conditions are preventable by proper cleaning of the eye and with eye drops [9]. In a systematic review of the literature best practice of eye care are stated in terms of procedures, care and products [6, 7]. Unfortunately, nurses participating in our study did not recognize the risks of iatrogenic eye infections. Only two quarters of nurses in cases of lagophtalmus would apply protective measures and nursing interventions such as: lubricant application, closing of the eyelids, ointment application every 4 hours. Number of nurses who did not know how to treat lagoftalmus or have no opinion about it was 6.3%.

Table 4. Answer distribution to questions from the domain of practice ECCI
Tabela 4. *Distribucija odgovora na pitanja iz domena prakse u Upitniku kliničke kompetencije za negu oka*

Type of care *Vrsta nege*	Always *Uvek*		Often *Često*		Occasionally *Ponekad*		Rarely *Retko*		Never *Nikad*	
	n	%	N	%	n	%	N	%	n	%
Washing hands before and after procedures *Pranje ruku pre i posle procedura*	83	87.4	7	7.4	2	2.1	3	3.2	-	-
Assessing and ensuring that patient's eyes are closed (on a daily basis)/*Proveriti i osigurati da su oči pacijenta zatvorene (dnevno)*	43	45.3	24	25.3	21	22.1	5	5.3	2	2.1
Assessing and ensuring that the endotracheal or the tracheostomy tube is correctly fixed in place (on a daily basis)/*Procena i osiguranje korektne fiksiranosti endotraheanog tubusa ili kanile traheostome (na dnevnoj bazi)*	42	44.2	21	22.1	19	20.0	6	6.3	7	7.4
Performing suctioning while standing beside, not above, the patient's bed and covering patient's eyes (PRN)/*Sprovođenje endotrahealne sukcije stojeći sa strane kreveta, ne iznad gornje ivice, uz pokrivanje očiju (po potrebi) (PRN)*	46	48.4	8	8.4	16	16.8	19	20.0	6	6.3
Cleaning the eyes by using normal saline or sterile water every two hours/*Prebrisavanje pacijentovih očiju fiziološkim rastvorom ili sterilnom vodom na svaka 2 sata*	28	29.5	13	13.7	28	29.5	14	14.7	12	12.6
Administrating appropriate eye lubricants every two hours *Primena lubrikanata za oči na svaka 2 sata*	14	14.7	14	14.7	20	21.1	19	20	28	29.5
Administrating appropriate eye drop every two hours *Primena odgovarajućih kapi za oko na svaka 2 sata*	18	18.9	17	17.9	19	20.0	27	28.4	14	14.7
Administrating appropriate eye ointment every four hours *Primena odgovarajuće masti za oko na svaka 2 sata*	18	18.9	24	25.3	21	22.1	17	17.9	15	15.8
Caring for each eye separately in case of unilateral eye infection *Nega za svako oko odvojeno u slučaju jednostrane infekcije očiju*	45	47.4	21	22.1	15	15.8	7	7.4	7	7.4
Using adhesive tape for closing patient's eyes appropriately/ *Korišćenje lepljive trake za zatvaranje pacijentovih očiju na odgovarajući način*	45	47.4	21	22.1	15	15.8	7	7.4	7	7.4

n = Absolute frequency/*Apsolutna frekvencija*; % = Relative frequency/*Relativna frekvencija*

Table 5. Correlation in knowledge, attitude and nursing practice domains on ECC test
Tabela 5. *Korelacija znanja, stava i prakse na Upitniku kliničke kompetencije za negu oka*

Variable/*Varijabla*	Knowledge/*Znanje*	Attitude/*Stav*	Practice/*Praksa*
Knowledge/*Znanje*	1	0.101	0.062
Attitude/*Stav*	0.101	1	0.529*
Practice/*Praksa*	0.062	0.529*	1

*p<0.01

Correct adhesive tape placement in order to close the eyelids and prevent that eyelashes damage eye surface is very important [9]. The horizontal placement of adhesive tape is recommended by the guidelines, but only 13.7% of nurses would do that correctly according to our study.

Eye health assessment should be a part of routine patient physical assessment practice in patients in intensive care units. During an eye health assessment one should perform: assessment of other eye structure, assessment of the white of the eye, and assessment of eye protective mechanisms [17, 18]. The results of our study show that nurses have the attitude that eye care is solely mechanical cleaning of the eye, and they are not aware of the importance of eye health assessment. Taking into consideration current knowledge level of nurses in our study considering eye care we can agree with Cho and al. that nurses in intensive care units do not have sufficient knowledge and experience in performing assessments for administering eye care [19].

Knowledge and attitudes are important for professional behaviours, because they have a major impact on nursing practices and interventions. Results from our study confirm this fact. Nurses with a more positive attitude toward eye care had higher scores in nursing practice domain. Nurses with higher educational level had better scores on knowledge domain. This correlates with Dowson study, which states that knowledge about the eye physiology and pathophysiology of eye damage increases nurse interest in intensive care toward eye care and prevention [7].

According to the best practice guidelines assessment of the eye closure should be performed at least once per shift, and after any nursing intervention and care for the eye [9]. Continuous evaluation and reevaluation of the eye makes prompt early diagnosis of iatrogenic eye conditions and early treatment possible. According to our study, 45% of the nurses make the assessment at the beginning of each shift, 25% of nurses perform assessment frequently, and 30% rarely or never perform the assessment.

The best nursing practices are under a constant process of evaluation, change and improvement. There is a need for further studies about eye care in intensive care units as a cornerstone for ongoing improvements in everyday nursing practices. According to our study data, nurses currently apply only basic principles and basic nursing practices in eye care, such as hand hygiene, asepsis and washing the eye with normal saline. Unfortunately, one third of our nurses do not apply eye washing with saline solution, or perform it very rarely. This corresponds with current literature data from Kam and collaborators [16]. Data from our study show that there is a lot of room for improvement in current nursing practices considering eye care, and that we need to follow evidence based nursing practices.

Conclusions

There is insufficient knowledge about some elements of nursing practices considering eye care, especially assessment and prevention of iatrogenic eye conditions. Low total score on tests in a nursing practice domain show that current nursing practices considering eye care are not in correlation with current evidence based knowledge. Taking into consideration moderate positive attitudes of nurses toward the eye care of patients on mechanical ventilation, there is a room for improvement of current nursing practices with the implementation of educational programs and making national guidelines for the best clinical practices on eye care in critically ill patients.

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NOVEL INFECTIOUS DISEASES IN EUROPE

NOVE INFEKTIVNE BOLESTI U EVROPI

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Summary

Introduction. The end of 20th and beginning of 21st century is marked by the discovery of new, supercontagious and fast spreading viral diseases. Since 1967, more than 40 new agents have been identified, including human immunodeficiency virus, Ebola, Marburg fever, severe acute respiratory syndrome, hepatitis C, hepatitis E viruses and Zika virus. Modern lifestyle, availability and speed of air traffic, migrations, as well as climate changes, enable faster spreading of infectious diseases from the regions that were hardly reachable. We selected a few diseases that raised the greatest attention among experts and public in general. **Ebola**. Ebola virus raises anxiety due to high mortality and fast spreading by using inter-human contact. **Zika virus.** Zika virus, that most often causes mild symptoms, is potentially responsible for microcephaly in neonates. **Dengue**. Dengue virus is an "old story", but in last decades incidence has multiplied by 30. **West Nile virus**. Although discovered in 1937, West Nile virus has been found exclusively in rural parts of Africa, while nowadays it represents one of the most important etiological factors of viral meningo-encephalitis all over the world. **Hepatitis E.** Today it is well-known that hepatitis E virus can cause not only acute viral hepatitis but also potentially blood-transmitted chronic hepatitis in immunocompromised, as well as some neurological disorders. **Conclusion**. One of the scientific challenges in the future will certainly be the discovery of available and cost-effective diagnostic tests, as well as efficient and safe vaccines for these diseases. Up to now, efficient prophylaxis is available only for Denga virus.

Key words: Virus Diseases; Hemorrhagic Fever, Ebola; Zika Virus Infection; Hepatitis E; Dengue; West Nile Fever; Global Health; Communicable Diseases, Emerging; Internationality; Epidemiology

Sažetak

Uvod. Kraj XX i početak XXI veka beleži otkriće novih virusnih bolesti, koje se pojavljuju u do sada nezapamćenim razmerama i veoma se brzo šire. Od 1967. godine, otkriveno je najmanje 40 novih uzročnika bolesti, uključujući HIV, ebolu, marburšku groznicu, SARS, hepatitis C, hepatitis E virus i zika virus. Moderni uslovi života, dostupnost i brzina aviosaobraćaja, sve veće migracije stanovništva, kao i promena klimatskih uslova omogućuju i daleko brži prenos infektivnih bolesti iz regiona na koje su pre navedenih globalnih promena bile ograničene. Izdvojili smo neke koje su unazad nekoliko godina privukle najveću pažnju kako stručne javnosti, tako i opšte populacije. **Ebola.** Virus ebole zebnju izaziva prvenstveno zbog visoke smrtnosti i kontaktnog puta širenja interhumano. **Zika virus.** Zika virus inače izaziva blagu kliničku sliku, a pažnju je privukao pre svega zbog potencijalnog razvoja mikrocefalije kod dece čije su majke zaražene ovim virusom. **Denga**. Sa virusom denge svetska javnost susretala se i ranije, ali je unazad nekoliko decenija zabeležen porast broja obolelih tridesetak puta. **Groznica Zapadnog Nila.** Iako otkriven još daleke 1937. godine, virus groznice Zapadnog Nila do skoro je bio ograničen na ruralne predele Afrike, a danas predstavlja jedan od najznačajnih uzročnika virusnih meningoencefalitisa širom sveta. **Hepatitis E.** Danas je poznato da virus hepatitisa E nije samo uzročnik akutnog virusnog hepatitisa nego i potencijalno krvnoprenosivog hroničnog hepatitisa kod imunokompromitovanih, kao i neuroloških oboljenja. **Zaključak**. Jedan od najvećih izazova nauke u narednom periodu svakako će biti pronalazak dostupnih i ekonomski opravdanih dijagnostičkih testova, kao i efikasnih i bezbednih vakcina protiv ovih virusnih bolesti. Za sada takva vakcina postoji samo za virus denge.

Ključne reči: virusne bolesti; ebola; zika virus; hepatitis E; denga; groznica zapadnog Nila; globalno zdravlje; zarazne bolesti u nastajanju; globalizacija; epidemiologija

Introduction

The end of the XXth and the beginning of XXIst century was marked by discovery of new viral diseases, which appeared in unseen proportions and spread briskly. Since 1967, at least 40 new pathogens have been discovered, including human immunodeficiency virus (HIV), Ebola, Marburg fever, severe acute respiratory syndrome (SARS), hepatitis C and hepatitis E virus and Zika virus. Other diseases priorly known, such as pandemic influenza, malaria, tuberculosis, leukemia, schistosomiasis, echinococcosis and many others, remained identically dangerous due to combination of mutations, growing resistance to drugs, problems with their eradication in developing countries and spreading to regions where they were almost unknown. The causes of the change in the epidemiological characteristics of infectious diseases during the XXth and XXIst centuries are effects of climate

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Abbreviations

HIV – human immunodeficiency virus
SARS – severe acute respiratory syndrome
WHO – World Health Organization
RNA – ribonucleic acid
PCR – polymerase chain reaction
WNV – West Nile virus
HEV – hepatitis E virus

changes (global warming, droughts, flooding etc.), urbanization coupled with the globalization of trade, travelling, wars, socio-economic factors and mass migrations of the population. The concept of globalization of infectious diseases, which includes interaction between man, vector and pathogen, is defined. The World Health Organization (WHO) is often declaring a global state of emergency due to the spread of infectious diseases from tropical regions to America and Europe (Ebola, Zika virus, etc.). In this article, we will outline only a few diseases that came into focus among the professional and the general public in our region.

Ebola

The virus of Ebola is a virus that caused probably the greatest public anxiety in recent years. It is a member of the *Filoviridae* family, recognized in the infectious society since 1976, when it was discovered as the cause of haemorrhagic fever epidemic near the Ebola River in the Democratic Republic of Congo. From the moment of discovery up until the end of 2014, the virus was only known to experts in the field of tropical medicine as the cause of sporadic hemorrhagic fever epidemics in Central Africa. None of these epidemics have exceeded the number of 425 infected patients by 2014 [1].

Ebola virus became a public health issue in March 2014, when an epidemic of Ebola was declared, rapidly taking on a global course, spreading to other continents. Retrospective research showed that the index case was recorded in Guinea [2] in December of 2013, but the epidemic was recognized and proclaimed in March 2014. When the end of the epidemic was declared in 2016, the highest number of cases was reached: 28,616 diseased and 11,310 deceased [1].

The great concern of both the professional and the general public is based on the following facts: the virus is highly contagious, transmitted by contact, clinical manifestation is extremely severe, presenting with febrile and hemorrhagic syndrome, the percentage of mortality is high (25–89% depending on the subtype of the virus), and there is no causal treatment, nor an adequate prevention.

The disease is transmitted by contact with the affected person or contaminated object, through blood and body fluids (urine, sweat, tears, stomach content, stool, breast milk and seminal fluid). It is still unknown for how long the virus is secreted with the seminal fluid of the person who survived the infection, as this can also be a potential route for transmission of the infection over a period which lasts longer than the duration of the longest incubation period, usually 2–21 days (10 days on average). A person who was in contact with an Ebola patient, and has no symptoms of the disease is not considered contagious [1].

Previous experiences with highly contagious viral diseases, that have severe clinical symptoms and a high mortality rate such as variola and measles, have shown that prevention on a global level is the best option for limiting the spread of the disease. Hence, currently the attention of research teams is focused primarily on prevention of the epidemic, instead of attempting to find an effective antiviral drug. Although there is still no registered and approved Ebola vaccine available for the time being, the most promising remedy is rVSV-ZEBOV (Rekombinant Vesicular Stomatitis Virus – Zaire Ebola Virus) vaccine, which is currently in phase two and three clinical trials [3, 4]. Namely, it is a virus-induced vesicular stomatitis that is obtained by a recombinant pathway, in which a single gene has been replaced by one of the genes from the Ebola virus genome. The live attenuated vesicular stomatitis virus replicates in the body and thereby initiates an immune response, which is also effective against Ebola viruses due to the addition of an individual gene from the Ebola virus genome. A single gene for the synthesis of an Ebola virus glycoprotein is not sufficient to cause Ebola. Phase 2 and 3 clinical studies, conducted on over 7,500 people who were in contact with Ebola patients, such as health workers who nursed and treated the ill and through other contacts, showed an efficiency of the vaccine from 75% to 100% depending on the regimen of administration [3], fine immunogenicity, and a satisfactory safety profile [4].

The last Ebola epidemic was recorded just a few months ago, in the period of May 11th–July 2nd, 2017 in the Democratic Republic of the Congo, with a total of 8 cases (suspected and/or proven Ebola virus disease), of which 5 were confirmed by laboratory. Four registered cases ended with a lethal outcome [1].

Zika virus Infection

In recent years, in many parts of the world, Zika virus infection has emerged as the new great health threat. In the decade following the discovery of Zika virus in Uganda in 1947, a large number of infected patients were registered in Africa, Asia, in the countries of South and Central America and the Caribbean. After an increased number of newborn babies with microcephaly and other neurological syndromes in regions with Zika virus in 2015, the WHO declared Zika viral infection an urgent problem of the international public health due to its possible association with microcephaly and other neurological syndromes [5, 6].

Zika virus is a ribonucleic acid (RNA) virus which belongs to the Flaviviridae family and is closely related to other viruses from this family - the Yellow Fever virus, Dengue, West Nile Fever virus, and

the Japanese Encephalitis virus. Zika virus is transmitted to people primarily through the bite of an infected *Aedes species* (*A. aegypti* and *A. albopictus*) mosquito that lives in tropical and subtropical climate zones. The virus may be transmitted vertically during pregnancy from the mother to the fetus. Additonally, individual cases of sexual and post transfusion transmission of Zika virus were described. The virus is isolated from breast milk, but no cases of disease acquired by breastfeeding have yet been reported [7].

The length of the incubation period for Zika infection is not precisely determined so far, but it is considered to be similar to other flavivirus infections transmitted by mosquitoes (lasting less than a week). Roughly 80% of cases of infection resolve without symptoms. If the disease manifests clinically, it is usually followed by elevated body temperature, itching maculopapular rash, polyarthralgia of small joints of the arm and feet, myalgia, conjunctivitis, retro bulbar pain and headache. Zika infection has a mild course, resolving in few days and rarely requiring hospitalization. Lately, some neurological syndromes, such as Guillain-Barré syndrome, have been linked to Zika virus infection, and the infection during pregnancy can lead to development of microcephaly in newborns [8, 9].

Diagnosis is based on clinical symptoms and laboratory tests. Serological tests for the detection of specific IgM antibodies are positive in a week after the onset of symptoms. Interpretation of these tests may be confounded by the occurrence of antibody cross reactions with other flaviviruses. Precise diagnosis is established by detection of Zika viral RNA (polymerase chain reaction (PCR) method), that has to be performed in the first week of disease due to a short term viremia. It is necessary to test patients with symptoms of the illness, who had spent the previous two weeks in regions where the presence of Zika virus was registered [7, 9].

Since there is no specific antiviral therapy for Zika virus infection, the treatment is solely symptomatic. There is no vaccine against the Zika virus infection available, and preventive measures consist of reducing the possibility (exposure) of getting this infection by avoiding mosquito bites and travel to regions with Zika virus [7, 9, 10].

Zika virus infection is a relatively mild disease and its major significance for public health is its association to neurological syndromes such as microcephaly and Guillain-Barré syndrome. Travels to the Zika infected regions and potential exposures to the infection, as well as the emerging climate changes, make the occurence of Zika viral infection likely in new regions such as Europe, too [11].

Dengue

Dengue is a tropical disease which belongs to the group of viral haemorrhagic fevers. During the past fifty years, the frequency of dengue has increased thirty times. Nowadays, dengue is present on all continents. In Europe, the first major outbreak of dengue fever occured at the end of 2016, on the Portuguese island of Madeira, during which 2,000 people developed the disease. Around 100 million infections and 25,000 deaths (mortality 4–5%) are registered each year worldwide [12, 13]. The cause of the infection is a Dengue virus from the genus *Flaviviridae*, of the *Togaviridae* family. Four serotypes are identified so far. After an infection with Dengue virus, person acquires a life-long immunity for a certain serotype and a short-term cross immunity. Infections with other serotypes of the virus increase the risk of developing more severe forms of the disease.

Dengue virus is transmitted to humans by bite of a female mosquito: *Aedes aegypti* (invasive species originating from Africa, with distribution to tropical and subtropical regions around the world) and *Aedes albopictus* (originating from Asia) [12, 14]. There is no interhuman transmission. The incubation period lasts for 4–15 days. The infective period starts shortly before the onset and lasts until the end of febrile state (6–7 days). After the infection, in most cases, the disease resolves asymptomatically. In 2009, the clinically manifested forms were classified by the WHO in two subtypes: an uncomplicated form and a severe form. An uncomplicated form of dengue infection is manifested by high temperature, shivering and fever, general algic syndrome and rash. The initial erythema of the face is the result of capillary dilatation and occurs immediately before or during the first two days of febrile state and morbilliform exanthema occurs in-between 3–7 days of febrility. Severe forms of the disease appear as dengue haemorrhagic fever and dengue shock syndrome and generally occur in people who have previously been infected with another serotype of the dengue virus. In these forms of the disease, after a febrile phase lasting 2–7 days, a brief drop in body temperature occurs and the disease passes to a critical phase. Large pleural effusions and ascites, severe bleeding from nose, gastrointestinal tract and lungs may occur due to an increased permeability of small blood vessels. A state of shock, multiple organ failure, and disseminated intravascular coagulation develops. Shock persists for a short period of time, usually 1–2 days. When a prompt supportive therapy is applied, the patient can recover rapidly [14, 15]. The initial laboratory findings of dengue are characterized by leukopenia, thrombocytopenia, metabolic acidosis and later hypoalbuminemia.

Diagnosis is established according to microbiological tests, isolation of the virus in cell cultures and detection of the nucleic sequences of the virus (PCR) in the early stages of the infection. It is reasonable to carry out serological detection of the antibodies only after the first week of the disease [16]. When dengue is in its initial stage, it is difficult to recognize the differences compared to other viral infections. During the period of rash, some other viral haemorrhag-

ic fevers, sepsis, malaria, leptospirosis, abdominal typhus, thrombocytopenic purpura should be taken into consideration as a possible differential diagnosis. The basic therapeutic approaches during the state of shock and prostration are based on timely intravenous fluid replacement (crystalloids). During refractory hypotension that does not respond to volume loading, colloids (dextran) and whole blood transfusions should be administered, followed by platelet and erythrocyte transfusions. The use of non-steroid anti-inflammatory drugs is not recommended, due to an increased risk of bleeding [17, 18]. In order to prevent the infection, the WHO recommends controlling the populations of mosquitoes and protection against their bites, and in endemic areas a vaccination with a tetravalent vaccine. The first tetravalent dengue vaccine was administered in Brazil, El Salvador, Mexico and the Philippines in December 2015. The vaccine is currently approved for people aged 9 to 45 years, and is given in three doses in intervals of 6 months between each dose. Since it is a live attenuated vaccine, its application is not recommended to be applied in immunocompromised individuals and individuals on steroid, immunosuppressive and chemotherapy [19].

The global goal of the WHO, set up in 2012, is to achieve a reduction in dengue mortality by at least 50% until 2020 and morbidity by at least 25% [12, 13, 19].

West Nile virus Infections

West Nile Virus (WNV) is a neurovirulent arbovirus belonging to the *Flaviviridae* family. It was first isolated from the blood of a febrile woman in Uganda (West Nile district) in 1937, which led to the name "West Nile fever" [20, 21].

The virus naturally exists in an enzootic cycle between birds as hosts and mosquitoes (*Culex pipiens, Aedes, Anopheles*) as vectors, with the occasional infection of humans, horses and other vertebrates [22, 23]. The first sporadic cases of WNV infections were limited to rural areas of Africa, but today WNV is the leading cause of meningoencephalitis in the United States, Europe and Australia. The WNV infection was officially registered in the human population for the first time on the territory of Republic of Serbia in late July and early August 2012, and since then it has been spreading steadily and increasingly. The virus is transmitted to humans by a bite of an infected mosquito, but it is also possible to intermittently transmit the infection, through transfusion of blood and blood products, organ transplantation and transplacentally. WNV was detected in human milk, but the transmission of viruses via breastfeeding has not been sufficiently investigated [23].

Approximately 80% of the infections in humans remain asymptomatic, in 20% there is an elevated body temperature that is most often followed by a rash, and in less than 1% of patients infection manifests as neuroinvasive disease such as meningoencephalitis, polyradiculoneuritis and acute flaccid paralysis [22–24]. Immunocompromised, patients with malignant diseases, elderly patients, people with diabetes and people who abuse alcohol are at greater risk for developing a neuroinvasive form of the disease. The West Nile fever is most commonly a spontaneously resolving disease of a favorable outcome. The neuroinvasive form of WNV infection is a severe disease with a fatal outcome in 4.2 to 18.6% of patients [20]. Complications of meningoencephalitis caused by WNV occur in 70% of patients in a form of long-term muscular weakness, movement disorders and cognitive impairment [20, 21].

The diagnosing of West Nile fever is established on the basis of detection specific IgM antibodies on serological tests, obtained from serum or cerebrospinal fluid. Early sampling within 7 days of the onset of the disease can show false negative results, thus it is of vital importance to repeat the analysis after 2–3 weeks, when the findings become positive in more than 98% of cases [24]. Early diagnosis is established by the detection of the RNA of the virus from blood and cerebrospinal fluid. In the neuroinvasive form of the disease, abnormalities of brain parenchyma and cerebellum may be visible on magnetic resonance, in roughly 70% of the cases [24].

The treatment of WNV is mainly symptomatic, since there is no specific antiviral drug. Some reports pointed out a possible beneficial effect of ribavirin and interferon alfa-2b [22, 23].

Preventive measures for WNV are not available for the time being, so prevention generally consists of general and individual actions against mosquito bites. There is no approved human vaccine for WNV yet [25].

Hepatitis E virus Infection

When it was discovered in 1983, hepatititis E virus (HEV) was perceived as a cause of acute viral hepatitis transmissed by fecal-oral contact in the endemic region of Asia. Since the discovery of the virus, the raising knowledge about HEV has been developing in three directions [26].

From the epidemiological point of view, there are some differences between developing and developed countries. Genotypes 1 and 2, transmitted by feces, are threathening the population of developing countries. Developed countries, on the other hand, face HEV genotype 3 and 4, as zoonoses, the cause of minor, alimentary or contact epidemics with reservoires of infection (pigs, deer, rodents, birds and other wild and domestic animals) [27]. However, attention is drawn to the recent indices about the possibility of transmission of HEV via blood and blood derivatives. Thorough studies conducted in non-endemic zones, where the rate of seroprevalence of anti HEV among asymptomatic individuals was as high as 50%, raised a question about the safety of blood samples in voluntary blood donors [28]. Studies in Germany and England have confirmed the presence of RNA of viruses in donated blood samples (England 1/2848 voluntary blood donors, German 0.08% voluntary blood donors)

and identified some undoubtable posttransfusion acute HEV in those who received blood products [29, 30].

From a clinical point of view, acute hepatitis E, fecoorally or parenterally transmitted, is defined as a self-limiting disease. Patients with pre-existing liver diseases and pregnant women should be observed with more attention, due to a higher rate of severe clinical forms of fulminant hepatitis [26]. Until recently, hepatitis E has been considered an exclusively self-limiting disease. However, now it is clear that patients after organ transplantation (both solid and stem cell transplatation), cancer patients, and HIV-infected (up to 60%) develop chronic hepatitis E after an asymptomatic acute, and most commonly post transfusion HEV [31]. Progression to cirrhosis is rapid and almost inevitable. For this reason, serological screening methods for the detection of anti HEV IgM and IgG, as well as PCR HEV RNA detection, became part of the obligatory algorithm for the diagnosis of liver lesions in the above mentioned patient groups. Therapeutic interventions are aimed mainly to reduce the dose of immunosuppressive drugs with administration of the ribavirin [27]. HEV can be the cause of neurological disorders in acute and chronic forms as well - Parsonage Turner's Sy, Guilain-Barré Sy, meningoradiculitis and multiple neuropathy.

When observed from the pathophysiological point of view, the diseases are HEV induced and immunologically mediated, even though in the case of meningoradiculitis a direct viral neurotrophic effect has been demonstrated. In neurological forms of HEV infection, hepatitis is asymptomatic, corroborated only by elevated aminotransferases in laboratory findings [32].

From the standpoint of prevention, in addition to territorially determined general prevention measures, the awareness of possible post-transfusion HEV identified the need for finding reliable, cost-effective screening tests for blood donors, as well as organ donors [30, 31]. Since the progress of modern medicine indicates that biological therapy will represent the backbone of a large number of diseases in the future, the question of the need for an adequate vaccine arises [32].

Conclusion

Having in mind all of the foregoing, one of the future challenges of science will certainly be to find affordable and economically cost-effective diagnostic tests for these viral infections, as well as to find effective and safe vaccines against these viral diseases, since the only currently available is for the Dengue virus.

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HISTORY OF CLINIC OF UROLOGY, CLINICAL CENTER OF VOJVODINA

ISTORIJA KLINIKE ZA UROLOGIJU KLINIČKOG CENTRA VOJVODINE

Goran MARUŠIĆ and Saša VOJINOV

Summary
December 2017 is the 65th anniversary of the establishment of Urology Clinic of the Clinical Center of Vojvodina. It is a long enough period of time to accomplish high goals set by the Urology Division as far back as in 1952. For more than a half of the century, our urologists have been following modern tendencies in diagnostics and treatment of the diseases of urogenital system. Today, we can proudly say that diagnostic and therapeutic approach to urogenital diseases in our environment has evolved greatly over this period and does not lag behind the one that is accepted worldwide.
Key words: History of Medicine; History, 20th Century; History, 21st Century; Urology; Urologic Diseases; Clinical Competence; Hospitals; Physicians; Famous Persons; Societies, Scientific

Sažetak
Decembra 2017. navršava se 65 godine postojanja i rada Klinike za urologiju Kliničkog centra Vojvodine. Ovaj je period dovoljno dug za postizanje visokih ciljeva postavljenih od samog osnivanja Urološkog odeljenja daleke 1952. godine. Više od pola veka urolozi su prihvatali sve savremene tendencije u dijagnostici i lečenju oboljenja urogenitalnog trakta i danas se sa zadovoljstvom može tvrditi da se pristup ovim urološkim oboljenjima ne razlikuje u našoj sredini od onog koji je opšteprihvaćen u svetu.
Ključne reči: istorija medicine; istorija, 20. vek; istorija, 21. vek; urologija; urološka oboljenja; klinička kompetentnost; bolnice; lekari; poznate ličnosti; naučna društva

Foundation

The history of urology in Novi Sad dates back to 1948, when Dr. Kosta Popov, urologist at the II Surgical Division of the Main Provincial Hospital established the Urology Division **(Figure 1)**. In 1952, the Division was transformed to the Department of Urology as a part of the Department of Surgery. The Department Head was Dr. Dimitrije Jeremić **(Figure 2)**. The Department had a capacity of 25 beds in the basement level and was managed by urology specialist Dr. Dimitrije Jeremić and resident Dr. Kosta Janča. Nursing services were provided by nuns. In 1954, the Department obtained 25 more beds and 197 surgical procedures were performed while still in the basement.

In 1956, the Department had 3 urology specialists (Dr. Dimitrije Jeremić, Dr. Kosta Janča, and Dr. Milenko Berić) and one resident (Dr. Borivoje Janković) **(Figure 3)**.

In 1964, after construction of the Clinic for Internal Diseases, the Department of Urology moved to a new building, which still is its current location, and was transformed to the department of the Clinic for Surgical Diseases **(Figure 4)**.

In 1970, Dialysis Division was established at the Department of Urology as one of the first of its kind in the country. Dialysis facility was built in 1972 including two urology operating rooms on the top floor. Pediatric urology had been practiced concurrently with the adult urology until 1973, when the Department of Pediatric Urology was established at the Clinic for Pediatric Surgery. In 1977, 10 physicians

Figure 1. Dr. Kosta Popov
Slika 1. *Dr Kosta Popov*

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Figure 2. Prof. Dr. Dimitrije Jeremić
Slika 2. *Prof. dr Dimitrije Jeremić*

were employed at the Department (Prof. Dr. Dimitrije Jeremić, Head of the Department, Dr. Kosta Janča – Assistant Professor, Dr. Milenko Berić– Assistant Professor, Prim Dr. Borivoje Janković, Dr. Miodrag Stojić, Dr. Aleksandar Bondarenko, Dr. Jovan Stojkov, Dr. Dimitrije Ristić, Dr. Dušan Bonert, and Dr. Miroslav Negrojević) and 20 nurses. 448 patients underwent surgical procedures.

Clinic in XXth Century

In 1980, the Department of Urology was transformed to the Clinic of Urology as a part of Associated Surgical Clinics. The Head of the Clinic was

Figure 3. Department staff, 1956
Slika 3. *Odeljenje 1956. godine*

Figure 4. Clinic of Urology, Clinical Center of Vojvodina
Slika 4. *Klinika za urologiju, Klinički centar Vojvodine*

Prof. Dr. Kosta Janča **(Figure 5).** In this period, transurethral surgery had become a standard procedure in the routine practice and the first *percutaneous nephrolitholapaxy* was performed in 1985 as one of the first ones in our country and Europe as well. In 1986, the first two cadaveric renal transplants were performed with the participation and substantial contribution of urologists from our Clinic. A new era in the management of urolithiasis began when modern device for extracorporeal shock wave lithotripsy (ESWL) was provided in 1988.

Figure 5. Prof. Dr. Kosta Janča
Slika 5. *Prof. dr Kosta Janča*

Prof. Dr. Jovan Stojkov was the head of the Clinic from 1988 to 2003. **(Figure 6).** This period was char-

Figure 6. Prof. Dr. Jovan Stojkov
Slika 6. *Prof. dr Jovan Stojkov*

acterized by substantial improvements in the approach to urologic diseases, mainly in the field of oncologic surgery and radical surgery of renal, bladder and prostate carcinoma and also retroperitoneal lymphadenectomy in testicular tumors became routine procedure. With the cooperation of colleagues from the Institute of Oncology in Sremska Kamenica and pathologists, radiologists and specialists in other medical branches from the Clinical Center of Vojvodina the Uro-oncology Consilium has been established along with the Urodynamic Dispensary.

Clinic in XXIst Century

In 2003, Prof. Dr. Goran Marušić became the Head of the Clinic. Major renovation and improvement of infrastructure was performed in 2006, and the third operating room was put into service. Keeping up with the latest accomplishments and trends in the field of urology resulted in the introduction of laparoscopic procedures in 2009. New devices for ESWL and urodynamics (2009) dramatically improved the treatment outcomes in urological patients.

Nurses and medical technicians play an important role in the development of the Clinic of Urology. Specific education on nursing care of urological patients has been carried out and supervised by our Head Nurses: Kiš Ester 1953–1959; Marija Aleksijević 1959–1974; Milanka Ilić 1974–1998; Mirna Šaravanja 1998–2010; Marija Ferko 2010–2012 and Maja Kočetov since 2012 until present.

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The staff of the Clinic includes 13 specialist-urologists (Prof. Dr. Goran Marušić, Prof. Dr. Jasenko Đozić, Prof. Dr. Vuk Sekulić, Prof. Dr. Saša Vojinov, Asist. Prof. Jovo Bogdanović, Assist. Prof. Dr. Ivan Levakov, Assist. Prof. Dr. Srđan Živojinov, Assist. Prof. Dr. Dimitrije Jeremić, Prim. Dr. Dragan Grbić,. MSc. Dr. Mišo Dukić, Dr. Dragoslava Nikolin Bošnjaković, Dr. Nebojša Dejanović, Dr. Žarko Dimitrić) and 2 residents (Assist. Dr. Senjin Đozić, Assist. Dr. Mladen Popov) as well as 34 nurses. The capacity of the Clinic is 47 beds. In 2016 a total of 1,429 patients underwent surgery, 798 extracorpreal shock wave lithotripsy procedures were performed and 25,226 patients were examined at the Specialized Outpatient Department.

Teaching and Scientific Activity

Professional and scientific activities of both doctors and nurses are noteworthy. The doctors are members of national and international urology associations. They actively participate at international meetings and conferences, which results in a remarkable number of scientific publications in both national and international journals. Nurses are active members and participants of professional meetings in the field of nursing.

Our Clinic of Urology is proud of its academic staff that includes two full professors, two associate professors as well as three assistant professors and two teaching assistants, who actively teach undergraduate and postgraduate courses in Serbian and English language at the Faculty of Medicine in Novi Sad.

The Clinic of Urology has been the teaching hospital affiliated with the Faculty of Medicine of the University of Novi Sad since 1977. Throughout this period, professors from our Clinic have been appointed Heads of the Chair of Surgery - Prof. Dr. Milenko Berić (1980–1983) and Prof. Dr. Kosta Janča (1986–1988). Prof. Dr. Goran Marušić was the Vice Dean for International Relations and Foreign Students from 2009 to 2015.

The Clinic of Urology organized a range of scientific meetings and congresses with eminent lecturers and participants in the field of urology - Prof. Gironcoli (Italy), Prof. Galizio (Italy), Prof. Cibert (France), Prof. Piquert (France), Prof. Smart (USA), Prof. Couvelaire and Prof. Kuss (France), Prof. Blandy (United Kingdom), Prof. Schmidt (Germany), Prof. Gregoir (Belgium), Prof. Bracci (Italy), Prof. Van der Werf Messing (The Netherlands), Prof. Dragan, Prof. Miclea, Prof. Bucuras (Romania), Prof. Romic, Prof. Pajor (Hungary), Prof. Madersbacher (Austria), and Prof. Schurch (Switzerland).

ВЛАДА АП ВОЈВОДИНЕ · VAJDASÁG AT KORMÁNYA
GUVERNUL P.A. VOIVODINA · ВЛАДА АП ВОЙВОДИНИ
VLÁDA AP VOJVODINY · VLADA AP VOJVODINE

Government of Vojvodina Building in Novi Sad
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SIGNIFICANCE OF IgG AVIDITY TEST IN DIAGNOSIS OF WEST NILE VIRUS INFECTION

ZNAČAJ TESTA AVIDITETA IgG ANTITELA U POSTAVLJANJU DIJAGNOZE INFEKCIJE VIRUSOM ZAPADNOG NILA

Ivana HRNJAKOVIĆ CVJETKOVIĆ[1,2], Jelena RADOVANOV[2], Gordana KOVAČEVIĆ[2], Aleksandra PATIĆ[1,2], Nataša NIKOLIĆ[1,2] and Vesna MILOŠEVIĆ[1,2]

Summary

Introduction. Serological tests appear to be the method of choice for establishing the diagnosis in the late phase of West Nile virus infection. Long persistence of IgM antibodies against West Nile virus is described and may be a problem for determination of the time of acquisition of West Nile virus infection. The aim of the study was to estimate the significance of IgG avidity determination in establishing the diagnosis of West Nile virus infection. **Material and Methods.** In a study 56 serum samples seropositive against West Nile virus were included. 24 serum samples were collected in 2012 from healthy residents of South-Backa district and 32 serum samples were collected in 2014 from 124 patients suspected of having West Nile virus infection. Commercial enzyme-linked immunosorbent tests were used for the detection of West Nile virus-specific IgM and IgG antibodies and IgG avidity. **Results.** Out of 124 patients suspected of having West Nile virus infection, 32 (25.8%) were seropositive for West Nile virus antibodies. Acute infection was laboratory confirmed in 15 (46.9%) cases. All patients with acute infection were West Nile virus IgM positive, 13 (85%) were West Nile virus IgG positive, and 2 (15%) had a borderline result for West Nile virus IgG antibodies. Out of 32 seropositive patients the presence of IgM antibodies was determined in 22 (68.7%). In a group of samples with high IgG avidity values, 6 were IgM positive, while 8 were IgM negative. **Conclusion.** West Nile virus IgM and IgG antibody serological assays alone are not sufficient for the accurate and reliable diagnosis of WNV infection. West Nile virus IgG avidity testing is necessary to ensure the differential diagnosis of acute from past West Nile virus infection.

Key words: West Nile virus; West Nile Fever; Diagnosis; Immunoglobulin G; Immunoglobulin M; Antibody Affinity; Enzyme-Linked Immunosorbent Assay

Sažetak

Uvod. Serološki testovi su metoda izbora za postavljanje dijagnoze infekcije virusom Zapadnog Nila u kasnim stadijumima infekcije. Dugo održavanje IgM antitela protiv virusa Zapadnog Nila je opisano i može biti problem pri određivanju vremena infekcije. Cilj ispitivanja je bio da se proceni značaj testa određivanja aviditeta IgG protiv virusa Zapadnog Nila u postavljanju dijagnoze infekcije virusom Zapadnog Nila. **Materijal i metode.** U studiju je uključeno 56 seruma seropozitivnih na virus Zapadnog Nila. Dvadeset četiri seruma su prikupljena 2012. godine od zdravih stanovnika Južnobačkog okruga i 32 uzorka seruma prikupljena su od 124 pacijenta sumnjivih na infekciju virusom Zapadnog Nila. Dokazivanje IgM, IgG antitela protiv virusa Zapadnog Nila i određivanje aviditeta IgG antitela vršeno je komercijalnim imunoenzimskim testom. **Rezultati.** Od 124 pacijenta suspektna na infekciju virusom Zapadnog Nila, 32 (25,8%) bilo je seropozitivno na virus Zapadnog Nila. Akutna infekcija je potvrđena u 15 (46,9%) slučajeva. Kod svih pacijenata sa akutnom infekcijom dokazana su IgM antiela. Trinaest (85%) je bilo IgG pozitivno dok su dva (15%) imala granične vrednosti IgG anitela. Kod 32 seropozitivna pacijenta 22 (68,7%) dokazana su IgM antiela protiv virusa Zapadnog Nila. U grupi uzoraka sa visokim aviditetom, šest je bilo IgM pozitivno dok je osam bilo IgM negativno. **Zaključak.** Za postavljanje dijagnoze infekcije virusom Zapadnog Nila nisu dovoljni serološki testovi za dokazivanje IgM i IgG antitela. Zbog dugog održavanja IgM antitela nemoguće je odrediti vreme infekcije samo primenom seroloških testova. Testovi aviditeta su zato neophodni za postavljanje dijagnoze i razlikovanje akutne od ranije infekcije.

Ključne reči: virus zapadnog Nila; groznica zapadnog Nila; dijagnoza; imunoglobulin G; imunoglobulin M; aviditet antitela; ELISA

Introduction

West Nile virus (WNV) is an arthropod-borne, neurotropic virus, with zoonotic potential, that belongs to the family *Flaviviridae*, genus *Flavivirus*, Japanese encephalitis serocomplex [1]. The members of this serocomplex are also other neurovirulent viruses such as Murray Valley encephalitis virus, St. Louis encephalitis virus, or Usutu virus. WNV is maintained in nature in an enzootic transmission cycle between birds, that are the natural reservoirs of WNV, and ornithophilic mosquitoes that have a role of vectors. Humans are considered "dead-end" hosts for WNV, as the low level of viremia in mammals is usually not sufficient to be

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Abbreviations

WNV – West Nile virus
RNA – ribonucleic acid
RT-PCR – reverse transcription polymerase chain reaction
ELISA – enzyme-linked immunosorbent assay
RAI – relative acidity index
JEV – Japanese encephvalitis virus
JFV – Yellow fiver virus
TBEV – tick-borne encephalitis virus
PRNT – plaque reduction neutralization test

transmitted to mosquitoes. WNV is an important human pathogen. It is estimated that 20% of infected people develop clinical symptoms and in less than 1% of them severe neurological diseases, meningitis, encephalitis, or acute flaccid paralysis, are observed [2]. For decades, the virus was endemic in Africa, southern Asia, north Australia and in many of the warmer regions of Europe. During the past several decades human WNV infections were mainly associated with sporadic cases, and since only sporadic outbreaks were reported, WNV was not considered a serious human health threat. WNV was introduced into New York in 1999, rapidly spreading to the entire country. The virus has become endemic in the United States, with thousands of human cases, and hundreds of neuroinvasive disease cases reported annually. Over subsequent years virus expanded to Canada, Mexico and Caribbean [3]. Endemic circulation of WNV has also been reported in many European countries. Great attention was paid to the epidemic of neuroinvasive cases of WNV human infections in Bucharest, Romania in 1996 when 393 cases of encephalitis and 17 deaths were recorded in people over 50 years old [4].

Although a serological study conducted in rural part of Vojvodina community revealed seroreactivity to WNV in up to 3% of the human population [5], the virus was not detected until 2010. The first direct detection of WNV in Serbia was from Culex pipiens mosquito pool collected in Novi Sad in 2010 [6]. Serological investigation of horse serum samples [7] and molecular investigation in wild birds [8] indicated that WNV circulated among animals in Vojvodina. The first outbreak with human cases of neuroinvasive WNV disease in Serbia was registered in 2012 when 58 patients were hospitalized at the Clinic for Infectious and Tropical Diseases, Clinical Centre Serbia in Belgrade. 52 of these patients had neuroinvasive and 6 had a febrile form of the disease. A total of 35 patients had completely recovered, while in nine patients fatal outcome was recorded [9]. Since 2012, human cases were recorded every year in Serbia. During 2013 and 2014, 32 patients were diagnosed with a neuroinvasive form of WNV infection and were treated at the Clinic for Infectious Diseases of the Clinical Centre of Vojvodina. Full recovery was recorded in 87.5% and lethal outcome in 3.13% of them [10].

Several different tests have been developed for the laboratory diagnosis of WNV infections. In the early phase of illness, diagnosis is based on the detection of viral ribonucleic acid (RNA) by reverse transcription polymerase chain reaction (RT-PCR) assays, real-time RT-PCR, and nucleic acid sequenced-based amplification. Identification of the WNV RNA in the CSF or serum during the acute stage of neurological involvement is generally considered to be a confirmatory diagnostic parameter. Viral isolation from tissues, blood or cerebrospinal fluid on cell culture (such as Vero E6, RK-13, AP61 or C6/36) is usually unsuccessful even in the early stage of infection because of the low viral load in humans. Virus isolation must be performed under biosafety level 3 conditions (BSL 3).

However, serological tests appear to be the method of choice for establishing the diagnosis in the late phase of virus infection. Significant problems in serological diagnosis are cross reactions between members of *Flavivirus* genus due to antigenic similarity [11]. Vaccination against Japanese encephalitis virus (JEV) or Yellow fever virus (YFV) can yield false positive results in enzyme-linked immunosorbent assays (ELISA) (ELISA, Euroimmun, Luebeck, Germany) IgM test for WNV [12]. Long persistence of IgM antibodies against WNV is described [13], and may be a problem for determination of the time of acquisition of WNV infection. Avidity IgG test can be used to help distinguish a recently acquired from past WNV infection [14]. Avidity is the binding intensity of interactions between the antibody and antigen. At the beginning of infection antibodies of low avidity are produced. During infection, in the process of affinity maturation, the avidity of IgG antibodies increases progressively there after within few months (high avidity) [15]. In the aim of determination of the avidity, the same sample is tested twice, applying ELISA or indirect immunofluorescence test. Once the sample is treated with urea as denaturing factor and parallel sample is exposed to phosphate buffer. In the process of denaturation low avidity IgG antibodies will liberate from antigen and high avidity antibodies will remain attached [16].

The aim of the study was to estimate the significance of IgG avidity determination in establishing the diagnosis of WNV infection.

Material and Methods

Between June and December of 2014, in the Centre of Virology at the Institute of Public Health of Vojvodina, blood serum samples from 124 patients suspected of having WNV infection were tested for the presence of antibodies against WNV. In addition, 24 serum samples collected before June 2012, from healthy residents of South-Backa district who were referred to the Institute of Public Health of Vojvodina for preoperative examination and were WNV IgG positive, were also included in the study. Commercial ELISA were used for the detection of WNV-specific IgM and IgG antibodies. Testing,

calculation, and interpretation of the results were performed strictly following the instructions of the manufacturer. Results were evaluated semiquantitatively by calculating a ratio of the extinction value (optical density value - O.D.) of the patient sample over the extinction value of calibrator 2 which was included in the test. Results were considered as positive if the ratio was equal to or greater than 1.1, borderline if the ratio was between 0.8 and 1.1 and negative if the ratio was less than 0.8. For determination of IgG avidity commercial ELISA using urea as a denaturing factor was carried out as described by the manufacturer. Serum samples were tested in duplicate: in one well with phosphate buffer and in the other with urea treatment. A relative avidity index (RAI) was calculated and expressed as a percentage by dividing the OD values with and without urea treatment and interpreted as follows: <40% low RAI indicating acute infection; 40–60% borderline RAI indicating recent infection; > 60% high RAI indicating past WNV infection.

Results

Out of 124 patients suspected of having WNV infection, 32 (25.8%) were seropositive for WNV antibodies. Acute infection was laboratory confirmed in 15 (46.9%) cases, 3 (9.4%) patients had a recent infection, and 14 (43.7%) patients had past WNV infection. Results of serological testing and IgG avidity testing for serum samples of patients with acute or recent WNV infection are presented in **Table 1.** Blood samples were obtained from 12 patients 5 to 37 days after onset of infection, while for 6 patients the data regarding the duration of illness were not available. All acutely ill patients were IgM positive and had IgG antibodies of low avidity (RAI<40%). Avidity index values were in the range from 15.1% to 38.7%. Two patients with acute infection had borderline results for IgG antibodies in ELISA (0.93 and 1.02) and avidity testing showed low values of RAI (32.7 and 22.7). Only in 7 (21.9%) cases, acute infection was confirmed by seroconversion between acute and convalescent serum sample. Borderline avidity results indicating recently acquired WNV infection were observed in three patients. Among them, 1 had borderline (0.93) and 1 had a negative result for IgM antibodies. In a group of patients with no available information about the date of symptom onset, there were 3 cases of acute infection and 3 cases of recent infection indicated by RAI values between 40% and 60%.

Nine (28.1%) patients were IgM negative and one had borderline IgM result (0.93). Positive re-

Table 1. Results of IgM and IgG ELISA and IgG avidity assays in serum samples from patients with acute and recent WNV infection
Tabela 1. *Rezultati ELISA IgM i IgG i testa aviditeta IgG u uzorcima seruma pacijenata sa akutnom i nedavnom infekcijom virusom zapadnog Nila*

Patient number *Pacijent redni broj*	Days from symptom onset to sample collection *Dani od početka bolesti do uzorkovanja*	IgM antibody level (Ratio*) *IgM antitela Količnik**	IgG antibody level (Ratio*) *IgG antitela Količnik**	Relative Avidity Index of IgG antibodies (%) *Indeks aviditeta IgG antitela (%)*	Result interpretation *Interpretacija rezultata*
4.	5	2.9	1.1	25.5	Acute infection/*Akutna infekcija*
6.	5	4.4	2.2	25.1	Acute infection/*Akutna infekcija*
12.	8	3.4	2.4	18.6	Acute infection/*Akutna infekcija*
14.	9	4.1	1.02	22.7	Acute infection/*Akutna infekcija*
15.	14	1.5	0.93	32.7	Acute infection/*Akutna infekcija*
17.	14	2.7	2.4	19.8	Acute infection/*Akutna infekcija*
19.	25	4.2	2.2	15.1	Acute infection/*Akutna infekcija*
20.	27	2.3	3.3	21.2	Acute infection/*Akutna infekcija*
21.	26	3.1	2.6	34.4	Acute infection/*Akutna infekcija*
22.	26	3.8	3.0	38.7	Acute infection/*Akutna infekcija*
23.	18	3.8	1.9	17.5	Acute infection/*Akutna infekcija*
24.	37	4.4	3.3	21.03	Acute infection/*Akutna infekcija*
26.	ND**	4.3	1.6	24.3	Acute infection/*Akutna infekcija*
27.	ND**	0.93	3.6	50.9	Acute infection/*Akutna infekcija*
28.	ND**	3.4	1.5	54.2	Acute infection/*Akutna infekcija*
29.	ND**	5.04	3.7	17.6	Acute infection/*Akutna infekcija*
31.	ND**	2.7	2.5	22.9	Acute infection/*Akutna infekcija*
32.	ND**	<0.8	1.2	45.6	Acute infection/*Akutna infekcija*

*Ratio - sample O.D./calibrator O.D.; **ND - No data/**Količnik - uzorak O.D./kalibrator O.D.; **NP - Nema podatka*

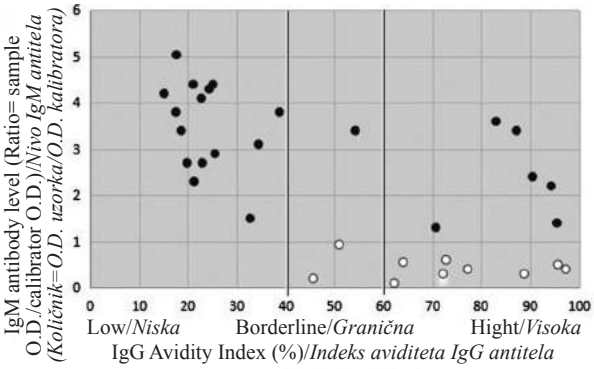

Figure 1. Results of IgM ELISA and IgG avidity test for serum samples of patients suspected of WNV infection
Grafikon 1. *Rezultati IgM ELISA i testa aviditeta IgG antitela u uzorcima seruma pacijenata suspektnih na infekciju virusom Zapadnog Nila*

sults for IgG antibodies were obtained in 27 (84.4%) cases, while 5 (15.6%) patients had borderline IgG results. As **Graph 1.** shows, all low IgG avidity serum samples were IgM positive. Among serum samples with borderline IgG avidity values, there was one positive, one negative, and one with borderline IgM result in ELISA. In a group of samples with high IgG avidity values, 6 were IgM positive, while 8 were IgM negative.

Results of IgG avidity test for serum samples of 24 healthy persons collected before the WNV epidemic 2012 are presented in **Graph 2**. Acute infection was confirmed in 1 (4.2%) case according to positive results for IgM and IgG antibodies in ELISA and low RAI (26%). Borderline values of RAI (49% and 55%) suggesting recent infection were found in 2 (8%) cases. The most seropositive healthy persons (22/25, 88%) had high RAI values, ranged from 68.4% to 98%, which was considered indicative of past exposure to WNV.

Values of RAI for 12 acutely ill patients with available data about the duration of illness are shown in **Graph 3**. Low avidity IgG antibodies were detected on the 5th day after symptom onset

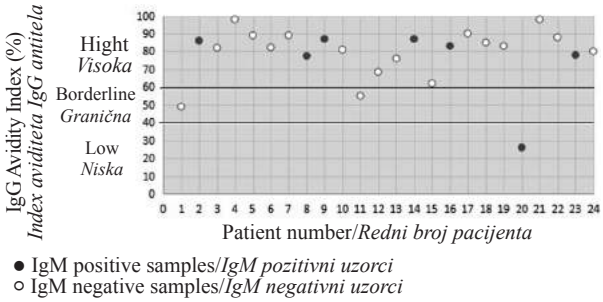

Figure 2. Results of IgG avidity test for serum samples of healthy persons collected before the WNV epidemic 2012
Slika 2. *Rezultati testa aviditeta IgG antitela u serumima zdravih osoba prikupljenim pre epidemije VZN 2012. godine*

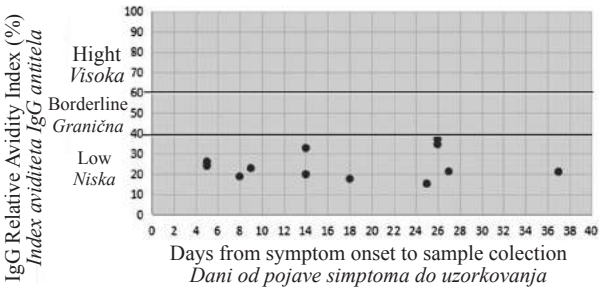

Figure 3. Results of IgG avidity test for serum samples of patients with acute WNV infection in relation to the time between onset of infection and sample colection
Slika 3. *Rezultati testa aviditeta IgG antitela za pacijente sa akutnom infekcijom izazvanom virusom Zapadnog Nila u odnosu na vreme proteklo od pojave simptoma do uzorkovanja seruma*

in 2 cases (RAI: 25.5% and 25.1%). In 75% (9/12) of patients from which the blood samples were obtained between 6 and 37 days after the onset of illness, RAI was lower than 25%, while the remaining 25% of patients had RAI higher than 30%.

Discussion

Modern molecular techniques allow the identification of WNV genome in serum and/or plasma samples from the 1st day of illness and have (been) demonstrated to be a powerful tool for early diagnosis of WNV infection. However, due to the short period of viremia molecular diagnostics is reliable only within the first few days of illness. Results of a study done by Busch et al. [17] indicated that WNV RNK was detectable, on the average, until 13.2 days post infection. Isolation of virus in cell cultures has the similarly limited significance in the diagnosis of WNV infection. It is useful only at the beginning of the infection. Moreover, isolation of WNV is time-consuming and requires the use of cell cultures and live, infectious virus so it must be performed under biosafety level 3 conditions which make it more cumbersome than the detection of WNV RNA by molecular assays.

Molecular and culture-based methods are applicable if the sample can be collected in the beginning of the infection, while WNV specific antibody detection in serum or liquor samples is the best approach for the diagnosis of WNV infection in later stages of illness. The most widely used serological method is ELISA because it is sensitive, easy to perform and can be fully automated which enables rapid simultaneous analysis of a large number of samples. Using ELISA, Prince et al. [18] discovered that WNV IgM and IgA antibodies can be detected 3 to 9 days and IgG antibodies 4 to 16 days, after the initial positive result for WNV RNK in blood. In our study, WNV IgM and IgG antibodies were determined in 22 (68.7%) patients suspected of having WNV infection and tested within 37 days from the onset of illness. However, IgG avidity testing confirmed acute infec-

tion in only 15 (46.9%) of them, while high IgG avidity test results indicated past infection in 6 (27.3%) cases. Diagnosis of past WNV infection was also established according to high IgG avidity test results (RAI >60%) in 8 WNV IgM negative patients. These results suggest that positive WNV IgM result is not always a proof of acute infection, which makes the diagnosis of WNV infection very difficult. The main reason for that is prolonged IgM response in WNV infected persons. Long-term persistence of WNV IgM antibodies, commonly for many months, is documented in a number of studies. Papa et al. detected low levels of WNV IgM antibodies in 3 out of 10 patients, 3 years after primary infection [13]. The presence of high avidity IgG antibodies in those 3 patients confirmed past infection. Busch et al. followed up 245 WNV RNK positive blood donors in order to assess WNV antibody development and persistence [17]. The mean time from the detection of WNV RNK to the detection of WNV antibodies in serum samples was 3.9 days for IgM and 7.7 days for IgG class. The mean time of antibody persistence was 156 days for IgM and 220 days for IgA antibodies. In a study of Roehrig et al., WNV IgM antibodies were determined in serum samples from 7 of 12 patients at approximately 500 days after onset of acute WNV encephalitis [19]. Results of an investigation done by Prince et al. indicated that specific IgM antibodies were detectable in 17%, IgA antibodies in 57% and IgG antibodies in 100% of 23 blood donors one year after the primary WNV infection [20]. Murray et al. also proved long-term persistence of WNV IgM antibodies. They confirmed the presence of WNV IgM antibodies 1, 6 and 8 years following acute infection in 42%, 34%, and 23% of patients, respectively [21]. Antibodies of IgM class do not cross the blood-brain barrier, so the presence of WNV IgM antibodies in cerebrospinal fluid strongly suggests acute central nervous system infection. However, there is some evidence of WNV IgM antibody persistence in cerebrospinal fluid from patients with neuroinvasive WNV infection, 110, 144 and 199 days after the onset of illness [22]. In addition to WNV IgM antibody persistence, data from animal models and patients who recovered from WNV encephalitis suggest that WNV not only causes acute disease but can also cause persistent infection. Prolonged excretion of WNV with urine (1.6 to 6.7 years) indicates replication of the virus in kidneys and persistent renal infection [23].

In our investigation, acute WNV infection was confirmed in 15 (46.9%) patients, while in 3 (9.4%) cases recent infection was demonstrated. All patients with acute infection were WNV IgM positive, 13 (85%) were WNV IgG positive, and 2 (15%) had a borderline result for WNV IgG antibodies. In all acutely ill patients, WNV IgG antibodies of low avidity were found with a RAI ranging from 15.1% to 37.8%. In 7 cases current infection was confirmed by the WNV IgM antibody detection in the acute-phase sample with seroconversion to IgG in a convalescent-phase sample. Documentation of seroconversion has been the "gold standard" for diagnosis of acute WNV infection. Taking into account that convalescent sample is not always available, IgG avidity testing is a useful tool for distinguishing current from recent or past infection in those cases.

Determination of the avidity of IgG antibodies may provide useful information regarding the timing of infection allowing differential diagnosis of acute from recurrent or past infection [24]. The IgG avidity test has been successfully used for the diagnosis of acute infections during pregnancy caused by teratogenic pathogens including protozoan parasite *Toxoplasma gondii* [25] and cytomegalovirus [26]. It has also been applied in the diagnosis of infections with flaviviruses such as WNV [27], tick-borne encephalitis virus (TBEV) [28] and Dengue viruses [29].

In this investigation, WNV IgM antibodies were determined in 7 of 25 (28%) WNV IgG positive healthy persons. Most of them (84%) had WNV IgG antibodies of high avidity. These findings are in agreement with other studies which have also documented WNV IgM antibody persistence over an extended period of time, indicating that WNV IgM antibodies are not a reliable marker of acute infection. It is estimated that approximately 80% of WNV infections are subclinical or asymptomatic [2]. In this study, acute asymptomatic infection was confirmed in 1 person with no clinical signs of illness.

Another problem that limits the clinical relevance of serological assays in WNV diagnostics is a high degree of cross-reactivity of antibodies produced in response to infection with different members of genus Flavivirus including St. Louis encephalitis virus, JEV, JFV, and Dengue 1–4 viruses. Antigenic similarity of the flavivirus envelope glycoprotein results in eliciting a cross-reactive antibody response and occurrence of false-positive results in serological assays [30]. A problem with misinterpretation of serological results is particularly prominent in geographic regions with co-circulation of various flaviviruses and implemented programs for vaccination against TBEV, YFV and/or JEV. The issue of cross-reactivity may be overcome by performing the plaque reduction neutralization test (PRNT) which has a high degree of specificity for target flaviviruses [31]. However, PRNT must be carried out under the biosafety level 3 conditions as a viable virus is used in this assay, and these facilities are available only in specialized research institutions not in routine diagnostic virology laboratories. Lack of PRNT as confirmatory assay was the limitation of this study. Immunofluorescence-based assays, such as "Flavivirus Profile 2" (Euroimmun, Luebeck, Germany) may be helpful in distinguishing infections caused by TBEV, WNV, JEV, YFV, and Dengue 1-4 viruses [32]. Recently, a new improved serological assay for discrimination of infections with WNV from those with other flaviviruses has been developed. It is based on differences in binding affinity to recombinant mutant E protein between WNV and other flaviviruses. An-

tibodies against WNV bound equally well to the wild type and the mutant type of protein, while antibodies from persons infected with TBEV and JEV show decreased affinity to mutant E protein [30].

Conclusion

The findings from a number of studies on West Nile virus IgM antibody persistence imply that serum IgM antibodies should not be considered a marker of acute West Nile virus infection. The results from our investigation concerning the detection of West Nile virus IgM antibodies in healthy persons as well as in patients with high avidity West Nile virus IgG antibodies, also suggest that the presence of West Nile virus IgM antibodies in serum samples do not necessarily indicate acute infection. Therefore, West Nile virus IgM and IgG antibody serological assays alone are not sufficient for the accurate and reliable diagnosis of West Nile virus infection. West Nile virus IgG avidity testing is necessary to ensure the differential diagnosis of acute from past West Nile virus infection.

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DEPARTMENT OF OTORHINOLARYNGOLOGY – FIFTY THREE YEARS SINCE THE ESTABLISHMENT

KATEDRA ZA OTORINOLARINGOLOGIJU – 53 GODINA POSTOJANJA

Slobodan M. MITROVIĆ, Zoran S. KOMAZEC and Rajko M. JOVIĆ

Summary

Otorhinolaryngology Clinic, which is an integral part of Vojvodina Clinical Centre, is the first teaching base of the Department of Otorhinolaryngology from 1964. It was founded only four years after the establishment of the Faculty of Medicine in Novi Sad, pursuant to the faculty's decision. From the beginning and until now ninety professors and assistants were their consistent part. The Department of Otorhinolaryngology joined the organization of the teaching process at the Faculty of Medicine, according to the Bologna Declaration's guidelines from 2005. Since this year 2017, the teachers of the Department of Otorhinolaryngology will conduct theoretical and practical teaching in ten teaching subjects in the Serbian language as a part of the first degree studies. For the purpose of taking specialist exams from 2016, the Department of Otorhinolaryngology has two specialist commissions, and from the previous period a commission for the examination of narrow specializations in audiology and phoniatry. The Department od Otorhinolaryngology celebrated 50th anniversary since its establishment in 2014 year. The Department of Otorhinolaryngology has been and now is a nursery for the best personnel in the field of Otorhinolaryngology in Serbia and in Vojvodina. Through the decades of its existence success can be seen through numerous student awards and recognitions which are the best proof for continuous work on acquiring new and expanding existing knowledge.

Key words: History of Medicine; History, 20th Century; History, 21st Century; Otolaryngology; Otorhinolaryngologic Diseases; Audiology; Clinical Competence; Hospitals; Physicians; Famous Persons; Education, Medical

Sažetak

Klinika za otorinolaringologiju, koja je sastavni deo Kliničkog centra Vojvodine, prva je nastavna baza Katedre za otorinolaringologiju osnovana 1964. godine, samo četiri godine nakon osnivanja Medicinskog fakulteta u Novom Sadu, na osnovu odluke Fakulteta. Od početka do sada, 19 profesora i asistenata nosioci su nastavne i naučne aktivnosti. Katedra za otorinolaringologiju se od 2005. godine uključila u organizaciju nastavnog procesa na Medicinskom fakultetu, u skladu sa uputstvima Bolonjske deklaracije. Od godine 2017. nastavnici Katedre za otorinolaringologiju izvode teorijsku i praktičnu nastavu iz 10 nastavnih predmeta na srpskom jeziku, na studijama prvog stepena. Da bi se omogućilo polaganje specijalističkih ispita, od 2016. godine Katedra za otorinolaringologiju ima dve specijalističke komisije a iz prethodnog perioda i komisije za polaganje ispita užih specijalizacija iz oblasti audiologije i fonijatrije. Katedra za otorinolaringologiju je proslavila 50 godina od osnivanja 2014. godine. Katedra je i sada rasadnik najboljih kadrova u oblasti otorinolaringologije u Srbiji i Vojvodini. Kroz decenije postojanja, uspeh se može videti kroz brojne studentske nagrade i priznanja koja su najbolji dokaz za kontinuirani rad na sticanju novih i širenju postojećeg znanja.

Ključne reči: istorija medicine; istorija, 20. vek; istorija, 21. vek; otorinolaringologija; otorinolaringološka oboljenja; audiologija; klinička kompetentnost; bolnice; lekari; poznate ličnosti; medicinsko obrazovanje

Introduction

Otorhinolaryngology Clinic, which is an integral part of Vojvodina Clinical Centre, is the first teaching base of the Department of Otorhinolaryngology since 1964. It was founded only four years after the establishment of the Faculty of Medicine in Novi Sad, pursuant to the faculty's decision.

The very beginnings of otorhinolaryngology are linked to a poet and a doctor Jovan Jovanović Zmaj. Doctor Nikola Matijević, who specialized in Ear, Nose and Throat in Berlin, started his first private practice in Novi Sad in 1923. In 1926 the ENT ward was founded in the city general hospital of Novi Sad and its Head was doctor Slobodan Matić. Doctor Vasa Krstić, assistant at the Faculty of Medicine in Zagreb, was a part of the ENT ward since 1927 as the ward's otolaryngologist. The Clinic was then moved to the ground level of the same building which was made in 1909. During Second World War, the ward's Head was Dionysus Denes Fodor, his position was then taken by doctor Andrija Hubert, followed by doctor Đorđe Fišer. After early death of Dr. Hubert, head position was assumed by Dr. Đorđe Fišer. When the Department of Otorhinolaryngology was established in 1964 Otorhinolaryngology ward was given the status of a Clinic. Dr. Đorđe Fišer was the first Head of Otorhinolaryngology Clinic and the first Head of Department of Otorhinolaryngology.

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Abbreviations
ENT – Ear, Nose and Throat

In the beginning of the school year 1976/77 the Faculty of Medicine was legally integrated with the core teaching units. Seventeen Basic Organizations of Associated Labour have been formed by a self-governing agreement, and one of them was the Clinic for Ear, Nose and Throat, as it was then named. In 1976 the Dental Department of the Medical Faculty was founded and the teaching became available for its students.

The Department of Otorhinolaryngology joined the organization of the teaching process at the Faculty of Medicine, according to the Bologna Declaration's guidelines from 2005. Now, the Department of Otorhinolaryngology can offer the following study programs: Integrated academic studies of medicine; integrated academic studies in dentistry; bachelor of special education and rehabilitation and bachelor of nursing. The classes are also conducted for students in English, students at second and third level studies as well as at specialist studies. Since 2017, the teachers of the Department of Otorhinolaryngology have started conducting theoretical and practical teaching in ten teaching subjects in the Serbian language as a part of the first degree studies. For the purpose of taking specialist exams from 2016, the Department of Otorhinolaryngology has two specialist commissions from the previous period, and a commission for the examination of narrow specializations in audiology and phoniatry.

The Department od Otorhinolaryngology celebrated 50th anniversary since its establishment in 2014.

The Heads of the Department of Otorhinolaryngology since its founding year

By 2017, the Department was led by six teachers at the Medical Faculty in the following election periods:

Prof. Dr. Đorđe Fišer
1964–1986.
Prof. Dr. Radivoj Topolac
1986–1990.
Prof. Dr. Dušan Milošević
1990–2001.
Prof. Dr. Rajko Jović
2001–2010.
Prof. Dr. Zoran Komazec
2010–2015.
Prof. Dr. Slobodan M. Mitrović
2015–present

From the Head ranks of the Department of Otorhinolaryngology, two teachers got appointed as Vice Deans, Prof. Dr. Dušan Milošević from 1980 to 1988 and Prof. Dr. Zoran Komazec since 2015.

Teachers and Associates of the Department of Otorhinolaryngology since 1964 to 2017

Professor Dr. Đorđe Fišer

The very first appointed Head of the Department was Prof. Dr. Đorđe Fišer who was the only lecturer for several years and who laid the foundations of otorhinolaryngology at the Faculty of Medicine, Novi Sad. Besides participation in undergraduate education for students of medicine and dentistry, during his engagement at the Department Prof. Dr. Đorđe Fišer made a significant contribution to the professional development of doctors ENT specialists. A great number of otolaryngologists of Vojvodina trained under his supervision. Prof. Dr. Fišer was a member of the commission for the examination of otorhinolaryngology at the Medical Faculty in Novi Sad and Belgrade. Dr. Fišer remained as Head of the Department until his retirement in 1986.

Figure 1. Teachers on 50th anniversary of Department of Otorhinolaryngology (October 2014)
Slika 1. *Nastavnici Katedre za otorinolaringologiju povodom 50. godišnjice (oktobar 2014.)*

Professor Dr. Radivoj Topolac

Since the establishment of the Faculty Prof. Dr. Radivoj Topolac was Prof. Fišer's associate. He was elected as an assistant at the subject of Otorhinolaryngology right after the establishment of the Faculty of Medicine in Novi Sad in 1961 and started with practical training three years later. Dr. Radivoj Topolac, a contemporary of modern microsurgery of ear - tympanoplasty, introduced this surgical method into the routine work of the Clinic for Ear, Nose and Throat. Prof. Radivoj Topolac took the helm of the Department in 1986 as a very experienced teacher and remained in that position until 1990. Even after his retirement Prof. Topolac remained close with the Department as a member of the Commission on professor elections and a reviewer of the first textbook of ENT Department "Otolaryngology – Head and Neck Surgery." As a member of the Medical Academy of Serbian Doctors' Society Prof. Topolac organized seminars as part of continuing medical education.

Professor Dr. Živko Majdevac

Upon his arrival at the Clinic of Ear, Nose and Throat in Novi Sad Dr. Živko Majdevac founded Phoniatrics Department and has made a significant contribution to the development of the Serbian phoniatrics. His work on voice and speech resulted in

a doctoral dissertation, which he defended in 1976 and then was elected in teaching positions. Prof. Živko Majdevac was the author of the original division of dysphonia and a very conscientious and meticulous educator to students of medicine and dentistry and physicians specialists. He was a member of the Department in 1993, when he retired.

Professor Dr. Dušan Milošević

After passing the Board Examination in 1970 Dr. Dušan Milošević got the position in practical training at the Department of Otorhinolaryngology, first as an associate and later as an assistant professor. He presented his doctoral dissertation in 1979 and through it, set the foundations for the teachings of vestibule functions in Yugoslavia and Novi Sad. After his doctoral dissertation Dr. Milošević was elected for teaching positions. As Vice Dean of the Medical Faculty in Novi Sad, in the period from 1980 to 1988, Prof. Dušan Milošević gave his significant contribution to the modernization and development of the teaching process at the Faculty of Medicine in Novi Sad. He was appointed Head of the Department of Otorhinolaryngology, Faculty of Medicine in Novi Sad in 1990 and remained at that point until his retirement in 2001. He was also appointed Head of the Department at the Faculty in Banja Luka in 1994. At that time Prof. Milošević was a mentor for four doctoral dissertations and twelve master theses which made a huge contribution to the academic development of the Department and ENT Clinics in Novi Sad and Banja Luka. Prof. Dušan Milošević was a longtime president of the Committee for Specialist examination in Otolaryngology at the Medical Faculty in Novi Sad and a member of numerous committees for post elections and defending of theses.

Professor Dr. Jelena Udovički

Right after passing a specialist exam in 1967 Prof. Jelena Udovički began working with students. She defended her doctoral dissertation, "Immunological changes in nasal secretions and serum of patients with nasal polyposis" at the University of Belgrade and was elected for the first teaching position in 1982. Professor Jelena Udovički was a responsible and devoted teacher of the Department and until her retirement in 1994 she conscientiously held student exercises. During her work at the clinic Prof. Jelena Udovički published dozens of papers and books primarily in the field of allergology and rhinopharyngology.

Assistant Professor Dr. Slavka Radić

Assist. Prof. Dr. Slavka Radić started working at the Department as an assistant in research work in 1977 when she also began with student practice. She was elected to the position of assistant professor after defending her doctoral thesis "Evaluation of olfactory and respiratory functions of the nose after rhinoplasty" in 1988. Assist. Prof. Radić was a member of the Department until she moved to private practice in 1994.

Assistant Dr. Dragana Levi

By becoming the teacher's assistant at the Faculty of Medicine in 1987, Dr. Dragana Levi began her work at the Department of Orohinolaryngology. She presented her master's thesis "The evaluation of auditory and vestibular functions in relation to the duration and progress of diabetes" in 1988. Dr. Dragana Levi, among other things, was also engaged in the development of contemporary audio logical methods, until her early leaving in 1991.

Professor Dr. Rajko Jović

After Prof. Milosević's retirement in 2001 a new Head of the Department was Prof. Dr. Rajko Jović who was elected as a teaching assistant in 1989. His doctoral dissertation was the result of his work on ENT oncology and the results of his dissertation greatly improved the quality of life of laryngectomized patients. After his doctoral dissertation Dr. Jović was elected for teaching positions. During his tenure Prof. Rajko Jović was intensively involved in improving education for physicians specializing in otolaryngology and establishing connections with many European ENT clinics. Prof. Jović also held lectures at the Faculty of Medicine in Banja Luka and Podgorica. He organized tenth International Courses of laryngomicroscopical surgery, two Courses and two Symposia of Head and Neck Surgery, and two Courses on the usage of ultrasound in diagnostics of head and neck pathology. In three scientific research projects he was a collaborator and author of several papers published in internationally acknowledged journals.

The specializations were done in the following institutions: "Vinogradska Hospital" , Zagreb, Croatia (1989), ENT Clinic, Szeged, Hungary (1989), "Šalata" Hospital Zagreb, Croatia (1991), ENT Clinic, Budapest, Hungary (1996), Krankenhause der Stadt Wien, Lainz, Vienna, Austria (2000), Krankenhause der Stadt Wien, Lainz, Vienna, Austria (2002), Vocal Protesis Training Course Amsterdam, Netherlands (2003), Laryngotracheal stenosis. Workshop. Manhaim, Germany (2006), Fifth European Course on Laryngology and Phonosurgery (2009).

Prof. Jović is the fourth Head of the Department of Otorhinolaryngology since its founding. He is the president of the First examination commission for the specialist examination in Otorinolaryngology and examination commission for Phoniatry. Prof. Jović is a President of Serbian ENT Society, and President of newly established Society of Serbian Head and Neck Oncology. He is a member of the Medical Academy of the Serbian Medical Society.

Professor Dr. Ljiljana Vlaški

By choosing to become an assistant in 1995, Dr. Ljiljana Vlaški started her work at the Department of Otorhinolaryngology, and participated in the practical teaching for students of medicine and dentistry even before being elected as an associate professor. She started her academic career as an assistant, at the Department of Histology and Embryology at the Faculty

of Medicine of the University of Novi Sad, in 1988, where she also obtained a master's degree in neuroendocrinology. After presenting her doctoral dissertation in 2001, she obtained a title of an assistant professor. Prof. Ljiljana Vlaški participated in the scientific-research project as the author of several papers in the field of children's otorhinolaryngology and otology. She teaches over 40 courses within the Continued Medical Education in the field of otology, otosurgery and pediatric otorhinolaryngology. She was the president of the Otorhinolaryngological Section at the Society of Physicians of Vojvodina of the Medical Society of Serbia from 2006 to 2010, and during that period she organized 8 thematic expert meetings. She was a mentor for students who did their master's thesis and doctoral dissertations. She is a member of the Commission where students can further their specialization in Otorhinolaryngology and Audiology.

Professor Dr. Dragan Dankuc

After being elected as an assistant in 1997 Dr. Dragan Dankuc began working at the Department of Otorhinolaryngology. He defended his doctoral thesis in 2001 after which he was elected in teaching positions. So far, Prof. Dragan Dankuc has held seven courses of continuing medical education dedicated to the dissection of the temporal bone and five courses devoted to microsurgery of ear. Also, he was the organizer of the Summer School of Otology. He is the Head of the Clinic for Ear, Nose and Throat of the Clinical Center of Vojvodina and a member of the Medical Academy of Serbian Medical Society.

Professor Dr. Zoran Komazec

Since 2009 Prof. Zoran Komazec, who was appointed as teaching assistant in 1997, has been elected the Head of the Department. His engagement in the physiology and pathology of the inner ear Dr. Komazec showed in his doctoral dissertation. Since his election for teaching positions Prof. Zoran Komazec has taught at the Faculty of Medicine and the Faculty of Technical Sciences in Novi Sad and the Faculty of Special Education and Rehabilitation, University of Belgrade. As a mentor, or a member of the Commission Prof. Zoran Komazec was engaged at the Faculty of Medicine, Nis and Faculty of Medicine, Foca - University of East Sarajevo. Prof. Komazec works on modernizing classes for students of medicine, dentistry and special rehabilitation and education. He is an associate in three scientific research projects and a mentor of numerous graduate and master's theses as well as a doctoral dissertation. He is a president of the Second commission for the specialist examination in Otolaryngology and the president of the commission for specialization examination -Audiology. He is the fifth Head of the Department of Otorhinolaryngology since its founding.

Professor Dr. Slobodan Mitrović

Dr. Slobodan Mitrović was elected as Assistant of the Department of Otorhinolaryngology in 1999. He presented his doctoral dissertation about the effect of opera singing in 2007, first in the Serbian medicine, after which he obtained the title of an assistant professor. Years of dealing with problems of laryngopharyngeal reflux, hereditary angioedema, chronic cough as well as physiology and pathology of singing, marked his work. In the project of the Faculty of Technical Sciences he worked as an associate on the development of a software for voice diagnostics. From this project the software was created that represents the original professional achievement of the author. He participated in two projects related to the development and progress of wider social communities, one of which was at the Medical Faculty of the University of Belgrade. He organized symposia of continuous education and developed publishing activity. He was also a member of the commission for the presentations of final works - graduate and master, as well as for doctoral dissertations. He is a member of the commission for the specialist examination in Otolaryngology and in Phoniatry. He is a reviewer in several medical and otorhinolaryngological journals. He is also the sixth Head of the Department of Otorhinolaryngology since its founding.

Professor Dr. Gordana Mumović

Dr. Gordana Mumović has been engaged at the Department of Otorhinolaryngology since 1995. She was elected as Assistant professor after defending her doctoral dissertation. As part of continual medical education, Prof. Dr. Gordana Mumović has held five courses in the field of diagnostic and therapeutic methods of voice and speech pathology. She has established cooperation with the Academy of Arts, University of Novi Sad, where she was a mentor of a doctoral dissertation. She is a member of the Commission for the specialist examination in Otolaryngology and in Phoniatry. She had a professional training at the Department of ENT, Phoniatrics section of Zagreb, Croatia (1990); European congress Budapest (1995); Professional voice seminar Athens, (1998); Salzburg Seminar (1998); City Hospital ENT Department in Vienna, Austria (2003); AKH- University Clinic – Phoniatric Department - Vienna, Austria (2005); Paneuropean Voice conference Marsell (2011); ENT Clinic Ljubljana – Slovenia (2013, 2016).

Professor Dr. Slobodan Savović

Dr. Slobodan Savović has been employed at the Department since 1997. After defending his doctoral thesis "Influence of septoplastics onto the course of chronic rhinosinusitis" in 2008, he was elected to teaching positions. As part of continual medical education, he organized two courses devoted to diseases of the nose and paranasal sinuses. He was a member of the Commission for position election of the Faculty of Medicine Military Medical Academy, University of Defence in Belgrade. He participated in a scientific research project and mentored undergraduate and master works, as well as a doctoral dissertation. He is a member of the Commission for the specialist examination in otolaryngology.

Professor Dr. Vladimir Kljajić

Dr. Vladimir Kljajić was appointed teaching assistant in 1997. After defending doctoral dissertation "The link of respirathory function and anthropometric characteristics of the nose and the face before and after rhinoseptoplasty" at the Faculty of Medical Sciences, University of Kragujevac in 2011, he was appointed assistant professor. He has been a mentor of defended graduate thesis. He is the author of several works in the field of laryngology and rhinology as well as three chapters in books in English. He is the current President of the Otorhinolaryngological Section at the Society of Physicians of Vojvodina of the Medical Society of Serbia.

Assistant professor Dr. Maja Buljčik Čupić

Dr. Maja Buljčik Čupić was elected to the position of a teaching assistant in 2003. She was elected assistant professor in 2012 after defending a doctoral dissertation. In addition to her engagement at the Department of Otorhinolaryngology assistant professor Dr. Maja Buljčik Čupić gives theoretical and practical lectures at the Department of Clinical Immunology. She is a member of the Commission for subspecialist examination in Allergology.

Assistant professor Dr. Slobodanka Lemajić Komazec

Dr. Slobodanka Lemajić Komazec was elected a teaching assistant in 1997. She defended her PhD thesis in 2012, after which she was appointed assistant professor. She has participated in a scientific research project of the Ministry of Science, Republic of Serbia as well as in the courses and symposia in the field of audiology and vestibulology. She has been a mentor of defended graduate thesis. She is a member of the Commission for subspecialist examination in Audiology.

Assistant professor Dr. Danijela Dragičević

Dr. Danijela Dragičević was elected associate teacher in 2007. After her PhD dissertation "Speech rehabilitation of totally laryngectomized patients with voice prosthesis insertion" which she defended in 2013 she was elected to the position of assistant professor. She has participated in national and international conferences on topics of laryngology and oncology.

Assistant professor Dr. Ljiljana Jovančević

Dr. Ljiljana Jovančević was elected assistant at the Department of Otorhinolaryngology in 2004. She defended her PhD thesis in 2013 after which she was elected to the position of assistant professor. As a lecturer, she held numerous lectures and workshops in the field of rhinology at domestic and international meetings: European Congress of Otorhinolaryngologists in 2007 in Vienna and 2013 in Nice; European Rhinological Congress in Amsterdam 2014; Croatian Rhinological Congress (2010, 2012, 2014, and 2016). She completed the course of Endoscopic Sinus Surgery in London in 2005 and Graz. Since 2015, she has been a secretary of the ENT Section of the SMC, as well as one of the founders, and the Secretary of the Rhinological and Allergological Assets of the ENT Section of SMC. She is a member of the Advisory Board of the European Association of Rhinologists (EAR) - as Serbia's representative in the board since 2016. She has been a member of the editorial board of the CCV magazine since 2017.

Doctoral Dissertations made at the Department of Otorhinolaryngology and presented at the Medical Faculty Novi Sad

The first doctoral dissertation was presented in 1972, and up to 2013, 17 doctoral dissertations were presented for all otorhinolaryngological disciplines: otology, rhinology, laryngology, audiology, phoniatry, as well as in the field of allergology and oncology.

1972.

Đorđe Fišer "Implementation of olfactometry and its clinical significance,"

Radivoj Topolac "Using alo, auto and homoplastic material in tympanoplasty "

1976.

Živko Majdevac "The human voice influenced by vocational burden on speech"

1979.

Dušan Milošević "Clinical vestibulometry in the diagnosis of head injuries"

1989.

Slavka Radić "Evaluation of olfactory and respiratory functions of the nose after rhinoplasty"

1998.

Rajko Jović „The impact of resection of laryngopharyngeal structures in laryngeal malignancy on swallowing act"

2001.

Ljiljana Vlaški "Clinical evaluation of the morphofunctional status of middle ear in surgically treated children with chronical noncholesteatomatous inflammatory processes",

Dragan Dankuc „The significance of tympanoplasty mobile bridge in surgical treatment of chronic middle ear diseases"

2004.

Zoran Komazec "Analysis of functional characteristics of medial olivocochlear system"

2007.

Slobodan M. Mitrović "Estimation of the opera singers` voice`s character with the morphoanthropometric parameters` analysis"

2008.

Dušanka Milošević "Clinical significance of the rhinoprovocation test in perennial non-allergic rhinitis."

Gordana Mumović „Therapy of the dysphonia after partial laryngectomy using larnyx compression"

Slobodan Savović "Influence of septoplastics onto the course of chronic rhinosinusitis"

2012.

Slobodanka Lemajić Komazec "Evaluation of the auditory and vestibular system in patients with multiple sclerosis"

Maja Buljčik Čupić „Determination of the effects of different therapeutic approaches in treatment of the sinonasal polyposis“
2013.
Danijela Dragičević „Speech rehabilitation of totally laryngectomized patients with voice prosthesis insertion”
Ljiljana Jovančević „Nasal nitric oxide concentration in diagnostics of inflammatory disorders of the nose and paranasal sinuses“

Continuous Medical Education

Since the very introduction of continuous medical education, the members of the Department of Otorhinolaryngology have been organizing scientific conferences. These meetings had educational purpose but they made an impact on the formation of the otorhinolaryngological profession in Serbia. Immediately after the establishment of the Center for Continuing Medical Education of the Faculty of Medicine, Prof. Dr. Zoran Komazec organized the second accredited course in this Center called “Cochlear implantation and rehabilitation of patients with cochlear implants” in December 2003. Moreover, he organized courses in “BAHA”, “Audiological Diagnostics” and “Tonal Audiometry.”

Prof. Dr. Ljiljana Vlaški organized the first accredited meeting of the Otorhinolaryngology Section in 2009, the Association of Doctors of Vojvodina-Serbian Medical Society, whose president she was then. Prof. Dr. Dragan Dankuc organized and directed educational seminars: “Time bone dissection”, continuously since 2007 and “Microsurgery of the middle and inner ear” since 2013. Under the auspices of the Academy of Medical Sciences SMS, Prof. Dr. Dragan Dankuc, in cooperation with other teachers and guest lecturers, organized the Summer School of Otorhinolaryngology from 2013 to 2016. Prof. Dr Rajko Jović, for many years, organized highly visited courses of continuous education: International Course “Laryngomicroscopic Surgery” since 2007 and “Head and neck surgery” seminar from 2010 to 2013; Course “Ultrasound diagnosis of head and neck inflammation” in 2013 and in 2015. Prof. Dr. Gordana Mumović organised “Diagnostic and therapeutic methods of pathology of voice and speech” (from 2009 to 2013) Prof. Dr. Slobodan M. Mitrović organized multidisciplinary symposiums:“Cough as a diagnostic and therapeutic problem”, “Laryngopharyngeal reflux”, “Disorders of function and esophageal diseases”, and “Edema and angioedema of the head and neck” and the symposium” Disorders of function and pharyngeal diseases” that will be presented in 2018. Prof. Dr. Vladimir Kljajić organized the “June ORL Symposium” in 2016 and the Symposium “Diseases of the ear” in 2017.

In addition to the teachers of the Department as organizers and lecturers, prominent otorhinolaryngologists and other experts from countries in the region, Europe and the world participated in the above mentioned meetings.

Members of the Department participated and continue to do so in the organization of numerous other meetings, from the meetings of the Section for Otorhinolaryngology of the Society of Physicians of Vojvodina and the Serbian Medical Society to the Congresses on national and international levels.

For self improvement, the teachers of the Department of Otorhinolaryngology were in numerous institutions in Europe and the world, with prominent experts in otorhinolaryngology and related disciplines.

Teacher Publishing Activity in the Department of Otorhinolaryngology

At the beginning of the 1980s, J. Udovički published his work in “Alergologic Immunological Handbook” and later in “Rare Diseases in Otorhinolaryngology” in two editions. After three editions of “Practicum of Otorinhinolaryngology”, by Ž. Majdevac, D. Milošević, R. Topolac and J. Udovički (1800 copies), the publishing activity of the teachers of the Department, marked three editions of the textbook “Otorhinolaryngology. Head and Neck Surgery,” by R. Jović (editor), Lj. Vlaški, D. Dankuc and Z. Komazec, printed in 18000 copies. After that, the “Questions collection from Otorhinolaryngology “ was published, which was compiled by all the teachers of the Department and have had two editions.

Lj. Vlaški is the author of the, until now, only National Guideline to good clinical practice in the field of Otorhinolaryngology, entitled “Otitis Media” published in 2004, under the auspices of the Ministry of Health and the European Union. Lj. Vlaški was active in implementation of this guideline.

R. Jović and Z. Fišer (DZ Novi Sad) published the monograph entitled “Emergency provision of the airway and the method of artificial ventilation.”

The monograph “Ethiology, Classification and Surgical Therapy of Disphony”, by S. Mitrović, was published by Andrejević Foundation from Belgrade. The monograph “Conservative Treatment of Dysphony” by G. Mumović, was published by the Faculty of Medicine. G. Mumović also published five collections of papers in electronic form called “Diagnostic and therapeutic methods of pathology of voice and speech”. S. Mitrović is the editor of the Proceedings “Hereditary Angioedema, Diagnostics, Therapy and Clinical Significance”, as well as four multidisciplinary symposiums that have been held so far. The publisher of these proceedings is the Society of Physicians of Vojvodina of the Medical Society of Serbia.

Also, under the auspices of the Department of Surgery, the authors S. Mitrović and M. Stanković, wrote the first textbook for the subject “Introduction to Clinical Practice I”, which until now had three editions in 1800 copies.

Authors R. Jović, G. Mumović, S. Mitrović in cooperation with S. Golubović (FASPER, Belgrade) published the textbook and practicum for the subject “Medical Basics of Voice and Speech Disorder”.

Teachers of the Department are the authors of the articles in numerous collections that followed the events they attended.

R. Jović wrote a chapter in the monograph "Medical Informatics" Mihaljev-Martinov (ed) (1995). With a group of teachers from the Department he was the author of a chapter in the monograph "Minimally Invasive and Endoscopic Head and Neck Surgery." Prgomet D. et al. (eds) (2010).

V. Kljajić, S. Savović and Lj. Vlaški are authors of chapters in three monographs of the renowned Springer Verlag Berlin Heidelberg publisher and it is in: Advanced Surgical Facial Rejuvenation. Erian A, Shiffman MA. (ed) (2012); Advanced Cosmetic Otoplasty. Shiffman MA (ed) (2013); Advanced Aesthetic Rhinoplasty. Shiffman MA, Di Giuseppe A. (ed) (2013).

Lj. Jovančević wrote a chapter in the monograph "Selected Topics in Toxicology". Vasović V. in 2009.

S. Mitrović and G. Mumović are the authors of the chapters in the monograph "Professional Voice" (2011) and G. Mumović in the "Laryngeal pseudotumors" (2013) both edited by V. Đukić.

It should also be noted that the teachers of the Department of Otorhinolaryngology are reviewers and members of Editorial Boards of reputable medical and otorhinolaryngological journals.

Conclusion

The Department of Otorhinolaryngology, from its founding until now, has been a nursery for the best personnel in the field of Otorhinolaryngology in Serbia and in Vojvodina. Success can be seen through numerous student awards, the recognition of the Faculty of Medicine "Cherry Flower," after the three-years successful ellection of the Department, for the best, continuous work on acquiring new and expanding existing knowledge, are the best proof of this, through the decades of its existence.

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FOURTEEN YEARS OF NEWBORN SCREENING FOR PHENYLKETONURIA IN VOJVODINA

ČETRNAEST GODINA NOVOROĐENAČKOG SKRININGA FENILKETONURIJE U VOJVODINI

Ivana KAVEČAN[1,2], Jadranka JOVANOVIĆ[1,2], Boris PRIVRODSKI[2], Milan OBRENOVIĆ[2] and Tatjana REDŽEK MUDRINIĆ[1,2]

Summary

Introduction. Phenylketonuria is an inborn disorder of metabolism, a rare, hereditary disease caused by deficiency of phenylalanine hydroxylase enzyme necessary for conversion of phenylalanine into tyrosine. The aim of this study is to determine the incidence of hyperphenylalaninemia and classical phenylketonuria in population of the Autonomous Province of Vojvodina. **Material and Methods**. We performed retrospective analysis at Medical Genetics Service, the Institute for Youth and Health Care of Vojvodina and examined the clinical material of the previous fourteen years, during the interval from 2003-2016. The analysis of the obtained results was carried out using descriptive statistics. **Results**. During fourteen years, from 01.01.2003 to 31.12.2016, 27 newborns with hyperphenylalaninemia were detected, and the incidence of hyperphenylalaninemia in the Autonomous Province of Vojvodina was 1: 9.525. Classical phenylketonuria was detected in 15 persons during indicated period, and the incidence was 1:17.143. **Conclusion.** Phenylketonuria is a hereditary disease whose adverse effects can be avoided, if it is recognized in time, and if recommended treatment measures are adequately applied, thereby improving the quality of life of persons affected by the disease as well as the whole family, that is facilitated by the introduction and implementation of neonatal screening.

Key words: Phenylketonurias; Phenylalanine; Neonatal Screening; Incidence; Epidemiology; Early Diagnosis; Infant, Newborn

Sažetak

Uvod. Fenilketonurija je urođeni poremećaj metabolizma, retko nasledno oboljenje nastalo usled nedostatka enzima fenilalanin hidroksilaze koji je neophodan za konverziju fenilalanina u tirozin. Cilj rada bio je utvrđivanje incidencije hiperfenilalaninemije i klasične fenilketonurije u populaciji Autonomne Pokrajine Vojvodine. **Materijal i metode**. Retrospektivnom analizom u Službi za medicinsku genetiku Instituta za zdravstvenu zaštitu dece i omladine Vojvodine obrađen je klinički materijal prethodnog četrnaestogodišnjeg perioda u vremenskom intervalu 2003–2016. godine. Analiza dobijenih rezultata sprovedena je metodama deskriptivne statistike. **Rezultati**. U četrnaestogodišnjem periodu 1.01.2003–31.12.2016. godine, detektovano je ukupno 27 novorođenčadi sa hiperfenilalaninemijom. Incidencija hiperfenilalaninemije u Autonomnoj Pokrajini Vojvodini iznosi 1 : 9.525. Klasična fenilketonurija je detektovana u navedenom periodu kod 15 osoba, a incidencija iznosi 1 : 17.143. **Zaključak**. Fenilketonurija je nasledno oboljenje, kod kog se mogu izbeći nepovoljne posledice bolesti ukoliko se na vreme prepozna i ukoliko se adekvatno primenjuju preporučene mere lečenja, čime se unapređuje kvalitet života osoba koje su obolele kao i cele porodice, što je omogućeno uvođenjem i sprovođenjem neonatalnog skrininga.

Ključne reči: fenilketonurija; fenilalanin; neonatalni skrining; incidenca; epidemiologija; rana dijagnoza; novorođenče

Introduction

Phenylketonuria (PKU) is the most common autosomal recessive inborn error of amino acid metabolism. If not diagnosed and treated, the amino acid phenylalanine (Phe) will accumulate due to deficiency of the enzyme phenylalanine hydroxylase (PAH), necessary to facilitate the hydroxylation of phenylalanine to tyrosine. In untreated patient phenylalanine accumulates in the body and leads to adverse toxic effects of a disease that are manifested as deterioration, seizures, microcephaly, mental retardation and other symptoms such as lighter hair, eyes and skin, the specific body odor, behavioral disorders, attention deficit and lack of concentration. There is a rare form of the disease (about 1–2%) caused by the deficiency of tetrahydrobiopterin (BH_4) cofactor [1–4].

Phenylketonuria was discovered by a physician - a biochemist from Norway, *Ivar Asbjørn Følling* in 1934. A gene encoding the phenylalanine hydroxylase synthesis is localized on the chromosome 12 (12q23.2) and consists of 13 exons. More than 950 mutations of this gene have been discovered so far [1, 4].

Depending on the degree of enzyme deficiency, disease can be divided in several forms: *Classical phenylketonuria* - the most severe, with the highest deficit of phenylalanine hydroxylase enzyme and phenylalanine concentration greater than 20 mg/dL (Phe > 1,200 mmol/L). *Moderate PKU* - there is residual enzyme activity, the concentration of phenylalanine is 15–20 mg/dL (Phe 900–1200 μmol/L). *Mild form* of *PKU* - there is a higher residual activity of the enzyme and the concentration of phenyla-

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Abbreviations

PKU – phenylketonuria
HPA – hyperphenylalaninemia
PAH – phenylalanine hydroxylase
Phe – phenylalanine
BH4 – tetrahydrobiopterin

lanine is 10–15 mg/dL (Phe 600–900 μmol/L). *Hyperphenylalemia* (HPA) - is a mildly elevated phenylalanine of 2–10 mg/dL (Phe 120–600 μmol/L).

The proposed classification according to the recommendations of the European guidelines is divided into two forms: A form that requires treatment to maintain a normal concentration of phenylalanine and form that does not need any treatment. Treatment is recommended for all cases in which the concentration of phenylalanine is over 6 mg% (Phe > 360 μmol/L) [4, 5].

Newly detected cases of phenylketonuria are most often detected through a newborn screening program. In the Autonomous Province of Vojvodina, a newborn screening program was introduced in 2003, and since then about 16 to 20 thousand newborns from 12 hospitals in Vojvodina are tested annually. During the year, one to four children with phenylketonuria and hyperphenylalaninemia are revealed. After the analyses are carried out, only children that have a phenylalanine concentration above the allowed limit repeat analyses. In the case of positive findings, a confirmation of the diagnosis is carried out at the Service for Medical Genetics. Parents receive all necessary information about the cause and nature of the disease, as well as about the possibilities of treatment and the necessary controls. Immediately after the confirmation of the diagnosis the treatment of the affected child begins, by introducing a low-protein diet with a low precisely defined intake of phenylalanine, which depends on the individual tolerance of phenylalanine. Treatment is necessary to start as soon as possible, ideally before the third week of the child's life, and at the latest by the end of the first month of life, allowing irreversible adverse consequences of the disease to be avoided and improving the quality of life of affected individuals and their families. Untreated disease leads to a gradual deterioration of intellectual function, microcephaly, epilepsy, autism, attention deficit, motor and behavioral disturbances and psychiatric symptoms [6–10].

Material and Methods

This study is retrospective analysis. We analyzed material of the previous fourteen years during 2003–2016 at the Department of Medical Genetics, the Institute for Children and Youth Health Care of Vojvodina. The study covered 257,157 initial analyses of the concentration of phenylalanine in newborns as well as additional confirmatory analyses. The source of data was the medical documentation. The total number of newborns by years was obtained from the Statistical Office of the Republic of Serbia. Biochemical analyzes were done using a Guthrie test and a fluorescent ninhydrin method. In the case with pathological concentration of phenylalanine, the parents of affected child have been informed about the nature of the disease and necessity of limiting the intake of phenylalanine through the implementation of a strictly controlled diet, which is initiated at the Department of Medical Genetics. Children affected by disease use special formulas and protein supplements with regular controls of phenylalanine levels. The analysis of the obtained results was carried out using descriptive statistics.

The aim of this study was to determine the epidemiological characteristics of phenylketonuria and hyperphenylalaninemia in the population of the Autonomous Province of Vojvodina.

Results

Department of Medical Genetics at the Institute for Children and Youth Health Care of Vojvodina is a regional institution that conducts neonatal screening program for the province of Vojvodina, with (according to the latest census) 1,931,809 inhabitants. The average number of live births changed and diminished over time, reaching 20,381 in 2003 and 17,107 in 2016. The total number of live births according to the data of the Statistical Office of the Republic of Serbia, for the fourteen years, from 1.1.2003. to 31.12.2016., was 257,157 **(Table 1)**.

The newborn screening program started in Vojvodina in 2003 and had been implemented for each newborn born in the province of Vojvodina. In a fourteen-year period from 01.01.2003 to 31.12.2016., 27 newborns with hyperphenylalaninemia were detected in total, and the incidence of hyperphenylalaninemia in the Autonomous Province of Vojvodina was 1 : 9,525. Classical phenylketonuria was detected during the specified period in 15 newborns, and the incidence was 1 : 17,143.

Classical phenylketonuria (> 20 mg / dL) was discovered in 15 newborns and moderate PKU (15–20 mg/dL) in 1 newborn. Mild PKU form was found (10–15 mg/dL) in 4 newborns, and hyperfenilalninemia (2–10 mg/dL) in 7 newborns **(Table 2)**.

The disease in all newly detected cases had been revealed in first three weeks of life and the treatment has been initiated immediately after the diagnosis was confirmed. The ratio of male-female sex in the test group was 1.3:1 (17 males and 10 females). Treatment begins if the values of phenylalanine are over 6 mg% (over 360 μmol/L), with the introduction of special formulas without phenylalanine and protein supplements.

Epilepsy was diagnosed in 2 children with hyperphenylalaninemia - moderate form (initial concentration of Phe was 16 mg%) and in one child with hyperphenylalaninemia (initial concentration of Phe was 7 mg%).

The association with other diseases was registered in one male child with a classical form of the disease

Table 1. Number of live births and the number of newly detected cases of hyperphenylalaninemia and phenylketonuria by years in the period 2003-2016.
Tabela 1. *Prikaz broja živorođenih i broja novootkrivenih slučajeva hiperfenilalaninemije i fenilketonurije po godinama u periodu 2003-2016. godina*

Year *Godina*	Live birth included by screening *Živorođeni obuhvaćeni skriningom*	HPA *HPA*	Mild form of PKU *Blaga forma PKU*	Moderate form of PKU *Umerena forma PKU*	Classical PKU *Klasična PKU*
2003.	20381				3
2004	20206				2
2005	19058		1		1
2006	19102		2		
2007	18380		2		
2008	18339	1			2
2009	18590				
2010	18145	2			1
2011	17410				1
2012	17932				
2013	17439				1
2014	17535	2			1
2015	17533	1			1
2016	17107			1	2
Total *Ukupno*	257.157	6	5	1	15

**HPA – hiperfenilalaninemija, PKU – fenilketonurija*

in the form of agenesis of one kidney. The male child with a classic form had an operation of cryptorchism.

Discussion

Phenylketonuria is inherited metabolic disease that, if detected prior manifestations of the disease and before elevated levels of phenylalanine toxically accumulate and affect the central nervous system, can be controlled by adequate intake of phenylalanine, so all adverse effects of the disease could be successfully avoided by treatment. In the period when there was no newborn screening program, the disease was detected after the child's parents observed deterioration and when the brain functions had already deteriorated and mental retardation developed. The introduction of the neonatal screening program was the success of the entire society. By implementing a special diet, children affected by the disease will not be affected by mental retardation and have all the conditions for enrollment and attendance at all levels of education. The effects of the newborn screening are largely reflected through the improvement of the quality of life, not just for the affected individual, but also for whole families and for the entire community [1, 5, 6, 11].

In previous fourteen years (2003–2016), in the territory of Vojvodina, 257,157 newborns were live born. Samples in the form of dry blood spots on filter paper were collected from maternity hospitals and health centers in Vojvodina (Pancevo, Ruma, Senta, Sombor, Sremska Mitrovica, Subotica, Backa Topola, Vrbas, Vrsac, Zrenjanin, Kikinda, Novi Knezevac, and Novi Sad) and sent at regular intervals to the Institute.

Table 2. The presence of certain forms of hyperphenylalaninemia and phenylketonuria in the population of Vojvodina depending on the concentration of phenylalanine in the blood prior initiation of treatment
Tabela 2. *Zastupljenost pojedinih formi hiperfenilalaninemije i fenilketonurije u populaciji Vojvodine u zavisnosti od vrednosti koncentracije fenilalanina u krvi pre započinjanja lečenja*

Form of PKU *Oblik fenilketonurije*	Phenylalanine concentration *Koncentracija fenilalanina*	Number of newborns *Broj novorođenčadi*
Classical form of PKU/*Klasična forma PKU*	>20 mg/dL	15
Moderate form of PKU/*Umerena forma PKU*	15-20 mg/dL	1
Mild form of PKU/*Blaga forma PKU*	10-15 mg/dL	4
Hyperphenylalaninemia/*Hiperfenilalaninemija*	2-10 mg/dL	7
Total/*Ukupno*		27

PKU – fenilketonurija

Figure 1. Territorial distribution of newborns affected by phenylketonuria and hyperphenilalaninemia in Vojvodina: Subotica, Futog, Begeč, Sombor, Ada, Sonta, Pančevo, Kovilj, Zrenjanin, Melenci, Noćaj, Kupusina, Mali Iđos, Vašice, Senta, Vrdnik, Alibunar, Sečanj, Srbobran, Mramorak.

Slika 1. *Teritorijalna distribucija novorođenčadi zahvaćenih fenilketonurijom i hiperfenilalaninemijom u Vojvodini: Subotica, Futog, Begeč, Sombor, Ada, Sonta, Pančevo, Kovilj, Zrenjanin, Melenci, Noćaj, Kupusina, Mali Iđos, Vašice, Senta, Vrdnik, Alibunar, Sečanj, Srbobran, Mramorak.*

If the value of phenylalanine in the blood was less than 6 mg%, the newborn did not require treatment. If the concentration of phenylalanine in the blood was between 360 μmol/L and 600 μmol/L (6 and 10 mg%), the treatment was performed up to 12 years of age. In the case of Phe concentrations over 10 mg%, lifelong treatments were recommended. In the fourteen-year period (2003–2016), 27 newborns with hyperphenylalaninemia were detected, out of which 15 had a classical form of the disease. Annually, one to four children have been discovered.

Treatment of PKU children should begin immediately after the confirmation of diagnosis, as every 4 weeks of delayed introduction of therapy reduces the IQ for 4 units. Immediately after establishing the diagnosis, the treatment of a child affected by the disease starts with the introduction of a low-protein diet with a low, precisely defined intake of phenylalanine and use of protein supplements. The outcome of the disease depends on the time elapsed from birth to the low phenylalanine diet introduction, and it is necessary to start treatment as soon as possible. It is ideal to start the treatment before the third week of life and at the latest by the end of the first month of life, thus avoiding the harmful effects of the disease and improving the quality of life of the affected people. All people with hyperphenylalaninemia are controlled at regular intervals, according to their age. Diet is reevaluated in relation to phenylalanine values in blood, age and body weight. Special forms of the disease are BH4 responders - when sapropterin treatment is applied [12–16].

Target concentrations of phenylalanine are 2–6 mg% for children aged 0–12 years and for maternal phenylketonuria, 2–10 mg% (120–600 μmol/L) for others (older than 12 years and non-gravid women).

Monitoring and treatment is planned according to age, compliance to the recommended diet, physical activity, the presence of acute infections and clinical status, along with the reevaluation of nutritional, clinical and biochemical status.

Early detection and treatment are necessary to avoid toxic effects of elevated phenylalanine, damage to the nervous system, growth failure, microcephaly, mental retardation, and neurobehavioral abnormalities.

The incidence of classical phenylketonuria in Vojvodina is 1:17,143, while the incidence of all hyperphenylalaninemias in Vojvodina is 1:9,525. The average incidence of PKU in the white race is from 1:10,000 to 1: 20,000. The variation between the geographic populations is high and the disease is more common in Turkey (1: 2,600), Iran (1: 3,300) and Ireland (1: 4,500). In Estonia it is represented by a medium incidence (1: 6,000), Tunisia (1: 7,600), and Slovenia (1:8.000). Incidence in Japan (1: 143.000), Finland (1: 200.000) and Thailand (1: 212.000) is low.

The expected incidence of sex is approximately equal, according to the autosomal recessive pattern of inheritance of the disease. The risk of a recurrence in a family when both parents are heterozygous is 25%. A prenatal diagnosis is possible by detecting the mutation from a sample of chorionic villi, amniotic fluid or from fetal blood.

Increased values of phenylalanine are toxic to the central nervous system. Exact mechanism of how phenylalanine impairs brain function is not clarified. The incidence of epilepsy in untreated forms of phenylketonuria is high. In untreated patients, the occurrence of West syndrome is significantly higher in relation to the general population. Although the adverse effects of the disease can be minimized by using an adequate diet, neuropsychological deficits with higher incidence are possible in comparison to the general population. When there are elevated levels of phenylalanine in the blood, the levels of monoamines are reduced: serotonin, dopamine and norepinephrine in the brain. Phenylalanine reduces the concentration of monoamine precursors. Tryptophan and tyrosine, according to the principle of competition for the transport of neutral amino acids, and also inhibits aminohydroxylases that play a role in the conversion of tryptophan and tyrosine to serotonin and norepinephrine. In untreated patients, the white brain mass is affected due to the toxic effect of phenylalanine on oligodendroglia. Even if the treatment starts immediately, there may be pathology of the white mass of the brain [17, 18]. In the analyzed group, two children had a registered epilepsy, one child from the group of moderate hyperphenylalemia, and another child from the hyperphenylalaninemia group.

In children affected by phenylketonuria, a higher incidence of congenital anomalies is not expected in comparison to the general population where congenital anomalies occur with a frequency of 3–5%. In the analyzed group, association with congenital anomalies was registered in one male child (3.7%; N = 1/27) with the classical form of the disease in the form of a kidney agenesis.

Conclusion

Phenylketonuria is inherited disorder of metabolism of the amino acid phenylalanine, whose adverse effects can be avoided by early recognition after the use of preventive neonatal screening. Early recognition and introduction of treatment prior to the manifestation of disease, improves the quality of life of people affected by the disease, as well as whole families and entire social communities. The incidence of classical phenylketonuria in the Autonomous Province of Vojvodina is 1 : 17.143, while the incidence of all hyperphenylalaninemias is 1 : 9.525.

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ACUTE MYELOID LEUKEMIA AND PROGNOSIS IN THE ERA OF MOLECULAR MARKERS

AKUTNA MIJELOIDNA LEUKEMIJA U ERI MOLEKULARNIH MARKERA

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Summary

Introduction. Acute myeloid leukemia is a malignant, clonal disease of a hematopoietic stem/progenitor cell, characterized by accumulation of acquired somatic genetic and epigenetic alterations. Acute myeloid leukemia is, basically, highly heterogeneous disease, so individual treatment approach is needed. With the use of high-throughput genome sequencing technologies, a full spectrum of recurrent gene mutations in acute myeloid leukemia has been discovered, which has also provided deeper insight into leukemogenesis and acute myeloid leukemia ontogeny. This review focuses on molecular markers with proven prognostic significance, which form, together with standard cytogenetics, basis for current acute myeloid leukemia risk stratification. Even in the era of molecular markers, standard prognostic factors (pre-treatment and postinduction factors) have a strong influence on the choice of postremission therapy. **Conclusion.** Evaluation of molecular markers and their impact on prognosis in acute myeloid leukemia should be interpreted in the context of complex gene interactions. Only comprehensive understanding of acute myeloid leukemia biology and integration of all prognostic markers enable us to timely plan risk adapted treatment.

Key words: Leukemia, Myeloid, Acute; Prognosis; Biomarkers, Tumor; Genetic Markers; Gene Expression Profiling; Mutation; Recurrence; Risk Factors

Sažetak

Uvod. Akutna mijeloidna leukemija je maligna, klonska bolest hematopoezne stem/progenitorne ćelije, koja nastaje kao posledica nakupljanja raznih stečenih somatskih mutacija na genetskom i epigenetskom nivou. Akutna mijeloidna leukemija je u osnovi veoma heterogeno oboljenje, što zahteva individualni pristup svakom bolesniku. Upotrebom savremenih sveobuhvatnih analiza genoma, otkrivena je čitava paleta molekularnih markera, što je takođe omogućilo dublji uvid u sam process leukemogeneze i ontogeneze ovog oboljenja. U radu su predstavljeni molekularni markeri sa već potvrđenim prognostičkim značajem, koji zajedno sa citogenetikom predstavljaju osnov savremenih podela akutne mijeloidne leukemije u odnosu na rizik. I u eri molekularnih markera, standardni prognostički faktori, pre započinjanja terapije i postindukcioni, bitno utiču na izbor postremisione terapije. **Zaključak**. Uticaj pojedinačnih molekularnih markera na prognozu akutne mijeloidne leukemije treba tumačiti u kontekstu drugih molekularnih markera. Samo dobro poznavanje biologije ovog oboljenja i integracija svih prognostičkih faktora omogućavaju blagovremeno planiranje terapije u odnosu na rizik.

Ključne reči: Akutna mijeloidna leukemija; prognoza; tumorski biomarkeri; genetski markeri; profilisanje ekspresije gena; mutacija; recidiv; faktori rizika

Introduction

Acute myeloid leukemia (AML) is a malignant, clonal disease of a hematopoietic stem/progenitor cell, characterized by accumulation of acquired somatic genetic and epigenetic alterations [1]. With the more accessible high-throughput genome sequencing technologies in a last decade, a full spectrum of recurrent gene mutations in AML has been discovered. Molecular heterogeneity of AML reflects complexity of the disease, with distinct clinical and biological entities. AML is now curable disease. Up to 40% of AML patients younger than 60 years of age can be cured, but in a term of diversity of the disease personalized approach to a risk adapted treatment is needed for every patient [2].

Integrated Genetic Profiling in AML and Functional Categories of Gene Mutations

The first AML genome was sequenced in 2008 and since then integrated genetic profiling in AML has revealed not only new recurrent gene mutations, but also patterns of co-mutations and mutual exclusivity, which put them in separate functional categories: mutations in signal transduction genes (FLT3, KIT, RAS), nucleophosmin 1, mutations in myeloid transcription factors (CEBPA, RUNX1), transcription factor fusions (PML-RARalfa, MYH11-CBFB, RUNX1-RUNX1T1), DNA modifiers (DNMT3A, IDH1/2, TET2), chromatin modifiers (ASXL1, MLL), tumor suppressors (TP53, WT1), cohesion complex (STAG) and spliceosome complex genes (SF3B1, U2AF1) [3] **(Table 1)**.

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Abbreviations

AML – acute myeloid leukemia
CN – cytogenetically normal
WHO – World Health Organization
ELN – European Leukemia Net
CR – complete remission

Beside classification of AML and related precursor neoplasm by the World Health Organization (WHO), which divides AML into distinct biological entities, based on genetics and clinical features, Papaemmanuil et al. established new genomic classification with eleven non-overlapping subgroups [4, 5]. This classification is based solely on genetic mutations and has emerged three new subgroups: chromatin-spliceosome, *TP53*-aneuploidy and provisionally, $IDH2^{R172}$ mutations. Comprehensive approach to molecular landscape of AML has made new perspective to AML leukemogenesis. At presentation, AML consists of several coexisting subclones, which have derived from so called founding leukemic clone [6]. Mutations in genes involved in epigenetics (DNMT3A, IDH, TET2, ASXL1) are considered as early events in leukemogenesis, in contrast to activating mutations (FLT3, RAS) which are late events and are frequently lost during disease progression [7]. Mutations in genes involved in epigenetics are "markers" of preleukemic clones, which can persist in longterm remissions [8]. Mutational patterns may be indicative of AML ontogeny (*de novo* versus secondary AML) [9, 10]. Mutations associated with secondary AML occur in genes involved in chromatin modifications, as well as in members of spliceosome and cohesin complex. In contrast, NPM1 mutations are relatively specific for *de novo* AML. *De novo* AML patients with this "secondary type" AML mutations share the same destiny as clinically diagnosed secondary AML [9]. AML genome has, in contrast to other malignancies (especially melanoma and lung cancer), low median mutation frequency [11]. Median number of mutations per AML patient is 4 and the number of recurrent mutations increases with age, which is in accordance with accumulation of somatic mutations [10]. Age related clonal hematopoiesis is an example of such stepwise mutations acquiring over many years, which portend greater risk for subsequent hematologic malignancies [12].

Molecular Markers With Proven Prognostic Significance

Standard cytogenetics or karyotyping is still the most important independent prognostic factor in AML [13]. The main disadvantage of conventional cytogenetics is relative low resolution (can miss cryptic rearrangements) and the fact that 45% of

Table 1. Functional categories of gene mutations
Tabela 1. *Funkcionalne kategorije mutacije gena*

Mechanism *Mehanizam*	Functional categories *Funkcionalne kategorije*	Gene mutations *Mutacije gena*
Induction of proliferation *Poremećaji proliferacije*	Activation of signalling pathways *Aktivacija signalnih puteva*	FLT3 mutation/*mutacija*, KIT mutation/*mutacija*, RAS mutation/*mutacija*
Nucleophosmin 1 mutation Nukleofozmin 1 mutacija		NPM1 mutation *NPM1 mutacija*
Disorders of differentiation *Poremećaji diferencijacije*	Transcription factor fusions *Translokacije koje pogađaju transkripcione faktore*	PML-RARA, MYH11-CBFB, RUNX1-RUNX1T1
	Mutations in myeloid transcription factors *Mutacije u transkripcionim faktorima*	RUNX1 (AML1), CEBPA, PU.1
Epigenetic changes *Epigenetske promene*	DNA methylation *DNK metilacija*	DNMT3A, IDH 1 and 2, TET *DNMT3A, IDH 1 i 2, TET*
	Histone modification/*Modifikacija histona*	MLL-X fusion, MLL-PTD, ASXL1, EZH2 *MLL-X fuzije, MLL-PTD, ASXL1, EZH2*
Other categories *Ostale kategorije*	Tumor suppresor genes/*Tumor supresor geni*	TP53, WT1, PHF6
	Cohesin complex genes *Kohezin kompleks geni*	STAG1, STAG2, SMC3
	Spliceosome complex genes *Splajsozom kompleks geni*	SF3B1, U2AF1, SRSF2

Legenda: FLT3 *(Fms-like tyrosine kinasa 3)*, RAS *(Rat Sarcoma viral oncogene homolog)*, NPM1 *(Nucleophosmin/nucleoplasmin family, member 1)*, PML *(Promyelocytic Leukaemia)*, RARA *(Retinoic Acid Receptor Alpha)*, MYH11 *(Myosin Heavy chain gene 11)*, CBFB *(Core Binding Factor Beta)*, RUNX1 *(runt related transcription factor 1)*, AML1 *(Acute Myeloid Leukaemia1)*, CEBPA *(CCAAT/Enhancer-Binding Protein gene α)*, DNMT3A (DNK metiltransferaza 3 A), IDH (isocitrate dehydrogenase), TET *(Ten-eleven translocation)*, MLL *(Mixed Lineage Leukaemia ili Myeloid-Lymphoid Leukaemia)*, PTD *(partial tandem duplication)*, ASXL1 *(additional sex combs like transcriptional regulator 1)*, EZH2 *(enhancer of zeste homolog 2)*, TP53 *(Tumour Protein p53)*, WT1 *(Wilms Tumour 1)*, PHF6 *(planthomeodomain finger protein 6)*, STAG *(stromal antigen)*, SMC3 *(Structural maintenance of chromosomes 3)*, SF3B1*(Splicing factor 3B subunit 1)*, U2AF1*(U2 small nuclear RNA auxiliary factor 1)*, SRSF2 *(serine/arginine-rich splicing factor 2).*

AML patients have cytogenetically normal AML (CN AML). The great majority of intermediate risk AML is CN AML, so molecular markers serve for further risk stratification [14]. Beside three mutations (NPM1, FLT3-ITD, CEBPA$^{double\ mut}$) with proven prognostic significance, mutations in several another genes (RUNX1, ASXL1, TP53) have emerged in the new European LeukemiaNet (ELN) risk stratification of AML [15].

Acute Myeloid Leukemia with Nucleophormin (NPM1) Mutation has been recognized as a new entity in the 2016 WHO classification. NPM1 mutation is one of the most common mutations in AML (up to 50% in CN AML). AML with NPM1 mutation, as a distinct clinicopathological entity, is usually associated with normal karyogram, leukocytosis, monocytic leukemias (French-American-British (FAB) M4 and M5a/b) and CD34 negativity [16]. Presence of NPM1 mutations in a patients with monocytic AML is associated with the development of leukemia cutis [17]. There is also a frequent cooccurrnce of NPM1 mutation with other molecular markers, especially with DNMT3A^{mut} and FLT3-ITD^{mut} [5, 18]. Prognostic significance of NPM 1 mutation depends on the presence or absence of other mutation(s). It is well known that NPM1 mutation in the absence of FLT3-ITD mutation or in the presence of FLT3-ITD low allelic ratio confers a favorable prognosis, which means that allogeneic stem cell transplantation is not indicated in the first complete remission [19, 20]. On the other hand, DNMT3A mutation has negative impact on prognosis among patients with CN AML with NPM1 mutation [21]. Surprisingly, NPM1^{mut} is considered to be secondary mutation, but in contrast to activating mutations in growth factor signaling pathways (for example FLT3 mutation), NPM1 mutation is relatively sustained during disease evolution and can be used as a good marker for the monitoring of minimal residual disease (MRD) [22].

Fms-Like Tyrosine Kinasa 3 (FLT3) mutation is frequent in AML, present in about one-third of patients. Two classes of mutations lead to constitutive activation of FLT3: internal tandem duplication (ITD) of the juxtamembrane (JM) domain and point mutation (usually D835) within tyrosine kinase domain (TKD). FLT3-ITD mutation, and not only the presence of the mutation but ITD allelic ratio, has proven dismal prognostic significance in AML [23]. Prognostic impact of the FLT3 mutant-to-wild type allelic ratio has been recently highlighted in the 2017 European LeukemiaNet risk stratification by genetics. At presentation, AML patients with FLT3-ITD mutation have high tumor burden with high, bone marrow and blood, blast percentage. AML with FLT3-ITD mutation is considered as a high risk leukemia, with increased relapse rate and reduced overall survival [24]. FLT-ITD mutation is frequently associated with NPM1 mutation and/or DNMT3A mutation. Recent data have shown that three way interactions also do matter. For example, presence or absence of FLT3-ITD^{mut} has pronounced effect on survival only in the context of concomitant NPM1^{mut} and DNMT3A^{mut} [6]. FLT3 is also a potential target for therapy. The multipotent kinase inhibitor, midostaurin, was approved in April 2017 for the treatment of adult patients with newly diagnosed AML who are FLT3 mutation-positive. Addition of midostaurin to the standard treatment of FLT3 mutation positive AML prolongs survival compared with placebo (RATIFY trial) [25].

CEBPA (CCAAT/Enhancer Binding Protein (C/EBP), alpha) is transcriptional factor, one of the key factors driving myeloid cell differentiation. While germline mutations are associated with familial cases of AML, somatic mutations occur in 7-22% of AML [26]. There are two types of CEBPA mutations: biallelic and monoallelic. Only CEBPA biallelic mutations have favorable impact on prognosis, so AML with CEBPA biallelic mutation is a new entity in the WHO 2016 classification [27]. AML with CEBPA biallelic mutation has a unique immunophenotype signature (combination of immature antigens with asynchronous maturation and aberrant CD7 positivity) and typically is not associated with NPM1 mutation and FLT3-ITD mutation [28].

Runt-Related Transcription Factor 1 (*RUNX1*), formerly known as AML1 is gene, which encodes alfa subunit of the core binding factor (CBF). AML with mutated RUNX1 is provisional entity in the new 2016 WHO classification. RUNX1 mutations are present in about 10% of AML, more frequent in older male patients and patients with wild-type NPM1 and CEBPA [29]. There is an association of RUNX1 mutation and immature morphology (minimally differentiated AML/M0 FAB). RUNX1 mutation is the most frequently mutated gene in the chromatin-spliceosome group and the most commonly co-occurring mutation is ASXL1 mutation. In contrast to favorable prognosis of RUNX1 gene fusions, i.e. AML with t(8;21), RUNX1 mutations portend worse prognosis in both younger and older patients with CN AML [30]. In contrast to somatic mutations, germline RUNX1 mutation is a unique subset of familial AML with preexisting platelet disorders and propensity to myeloid malignancy (estimated risk of up to 60% of developing myelodysplastic syndrome or AML) [31].

Additional Sex Combs Like-1 (ASXL1) mutation is present in about 10% of AML patients [10]. There is a progressive increase in incidence of ASXL1 mutation with age, which is in line with the association of ASXL1 mutation and myelodysplastic syndrome and the association of ASXL1 mutations with secondary AML. ASXL1 mutation is inversely associated with FLT3-ITD and NPM1 mutation [32]. On the other hand, there is co-occurrence with RUNX1 mutation, which has additive, prognostically unfavorable effect [33]. AML patients with *ASXL1* mutations have lower complete remission rates after standard chemotherapy and shorter survival [18, 32, 33].

Tumor Protein 53 (TP53) is a well known tumor suppressor. TP53 mutation is relatively rare in AML

(2-8% of cases) and is strongly associated with complex and monosomal karyotype and abnormalities of chromosome 5, 7, 17 [34]. AML patients with TP53 mutation are resistant to standard chemotherapy and this chemoresistance can be mitigated by the use of hypomethylating agents, like decitabine [35].

Acute myeloid leukemia with BCR-ABL1 is a provisional entity in the new WHO 2016 classification of AML. Translocation t(9;22)(q34.1;q11.2) is a hallmark of chronic myeloid leukemia and also well established, negative prognostic factor in acute lymphoblastic leukemia. There has been dilemma whether *de novo* Ph positive AML really exists or it is only blastic phase of chronic myeloid leukemia at presentation. Some clinical features speak more in favor of *de novo* AML (for example, less prominent splenomegaly, fewer peripheral basophils, decreased myeloid to erythroid ratio). In correlation with some cytogenetic (monosomy 7, chromosome 16 inversions and chromosome 10 deletions) and molecular characteristics (presence of NPM1 mutation as well as more frequent P190 form of *BCR/ABL*) the diagnosis of *de novo* Ph positive AML is more probable [36]. AML with BCR-ABL1 is a rare type of leukemia (around 1% of AML) with dismal prognosis [37]. Use of a target therapy with thyrosine kinase inhibitors in combination with standard chemotherapy and allogeneic stem cell transplantation in first complete remission seems to be the best option for AML patients with BCR-ABL1 [38].

Other Molecular Markers and an Integrated Approach to the Choice of Postremission Therapy

There is a whole list of other molecular markers, including DNMT3a, IDH 1, IDH 2, TET 2, etc. with potential prognostic significance, which are not yet recommended for routine everyday practice, but are incorporated in some prognostic models [18, 21, 39]. Integration of molecular markers with proven prognostic significance with the standard cytogenetics is a basis of the novel 2017 ELN risk stratification of AML, which divided patients into three distinct risk categories (favorable, intermediate and adverse) [15] **(Table 2)**.

Not only presence or absence of gene mutations, but overexpressions of some genes also have prognostic significance in AML. Overexpression of brain and acute leukemia gene, cytoplasmic (BAALC), as well as high expression of Ets related gene (ERG) or meningioma1 (MN1) gene, is associated with unfavorable prognosis [40, 41]. High *EVI1* expression is a strong negative prognostic factor in AML, even in the cases without associated inv(3) and t(3;3) or *MLL* rearrangement, as well known mechanisms of *EVI1* gene deregulation [42]. In our investigation, relative *EVI1* overexpression was observed in 13.2% of the patients, which is in line with other investigations [43, 44]. Our results confirmed high *EVI1* expression as a poor prognostic factor in AML and also showed inverse correlation of high *EVI1* expression and NPM1 mutation, and on the other hand significant association of high *EVI1* expression with monosomy 7 [43, 44]. Vast majority of AML patients with mono-

Table 2. 2017 ELN risk stratification by genetics (adapted from ref. 15)
Tabela 2. *2017 ELN podela u odnosu na rizik, bazirana na genetici (adaptirano iz ref. 15)*

Risk category *Kategorija u odnosu na rizik*	Genetic abnormality *Genetska abnormalnost*
Favorable *Dobra*	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD *low allelic ratio/*Mutirani *NPM1* bez *FLT3*-ITD ili sa *FLT3*-ITD *nizak odnos alela* Biallelic mutated *CEBPA/* Dvostruka *CEBPA* mutacija
Intermediate *Srednjeg rizika*	Mutated *NPM1* and *FLT3*-ITD*high allelic ratio/Mutirani NPM1 i FLT3-ITD visok odnos alela;* Wild type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD*low allelic ration* (without adverse risk genetic lesions)/*Nemutirani NPM1 bez FLT3-ITD ili sa FLT3-ITD nizak nivo alela (bez loših citogenetskih lezija);* t(9;11)(p21.3;q23.3); *MLLT3-KMT2A;* Cytogenetic abnormalities not classified as favorable or adverse/ citogenetske *Abnormalnosti koje nisu klasifikovane kao dobre ili loše*
Adverse *Loša*	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged/rearanžman *KMT2A* t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* -5 or del(5q); -7; -17/abn(17p) Complex karyotype, monosomal karyotype/kompleksni kariotip, monozomalni kariotip Wild type *NPM1* and *FLT3*-ITD*high allelic ratio/Nemutirani NPM1 i FLT3-ITD visok odnos alela* Mutated *RUNX1*/Mutirani *RUNX1* Mutated *ASXL1*/Mutirani *ASXL1* Mutated *TP53/* Mutirani *TP53*

somy 7 have high *EVI1* expression, irrespective to whether inv(3)/t(3;3) is present or not, and these patients have particularly dismal prognosis [44].

Even in the era of molecular markers in AML, standard prognostic factors (patient-related and disease-related) still play an important role in an integrated approach to the choice of postremission therapy [45]. Beside cytogenetic and molecular markers, other standard disease related factors are of special concern in predicting risk of relapse. These are: white blood cell count at presentation, prior history of myelodysplastic syndrome (secondary versus *de novo* AML), number of induction cycles required to achieve complete remission (early, versus late complete remission) [46]. Initial high white blood cell count (more than $100x10^9$/l), as an indicator of high tumor burden, as well as secondary AML is associated with a worse prognosis [46, 47]. Early blast clearance and achievement of CR (complete remission) after one induction cycle (early CR) are independent postinduction favorable prognostic factors in AML [48]. Monitoring of minimal residual disease (MRD) by real-time quantitative polymerase chain reaction (RT-qPCR) or multiparametar flowcytometry is another useful tool for relapse prediction and can be used for tailoring postremission therapy [22, 49]. For example, MRD negative AML patients with favorable and even intermediate risk can be candidates for autologous peripheral blood stem cell transplantation as an option for consolidation therapy [20]. Patient related factors, like age, comorbidities and performance status are closely related to nonrelapse, transplant related mortality and have significant influence on decision making about intensity of postremission therapy [50]. To summarize, decision whether to proceed to allogeneic hematopoietic stem cell transplantation (alloHSCT) in the first complete remission of AML or not is weighted between the risk of relapse following chemotherapy and the risk of relapse and nonrelapse mortality following alloHSCT [20, 45]. This is possible only by the integration of all above mentioned factors, with cytogenetics and molecular markers as cornerstones.

Conclusion

Whole genome sequencing technologies (next generation sequencing) are much more accessible, less time consuming and cheaper, and now are not reserved only for research. Conventional cytogenetics is still the most important independent prognostic factor in acute myeloid leukemia, but more and more genetic mutations are with proven prognostic significance. Evaluation of molecular markers and their impact on prognosis in acute myeloid leukemia should be interpreted in the context of complex gene interactions. No single prognostic factor is sufficient to make a proper risk stratification of cytogenetic patients, so integration of standard clinical, acute myeloid leukemia and molecular factors is the best approach. Clinicians need a single, next generation sequencing based test at diagnosis, which can be substitute for several standard tests like karyotype, fluorescence *in situ* hibridisation) and polymerase chain reaction, to comprehend biology of each acute myeloid leukemia and timely plan risk adapted, individualized treatment.

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EPIDEMIOLOGICAL CHARACTERISTICS OF OCCUPATIONAL ANTHROPOZOONOSES IN THE AUTONOMOUS PROVINCE OF VOJVODINA

EPIDEMIOLOŠKE KARAKTERISTIKE PROFESIONALNIH ANTROPOZOONOZA U AUTONOMNOJ POKRAJINI VOJVODINI

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Summary

Introduction. Anthropozoonoses are diseases that affect humans and animals and they can be transmitted naturally from animals to humans. The aim of this study was to identify characteristics and types of occupational hazards that lead to occupational anthropozoonoses, determine their incidence in economic activities of the Autonomous Province of Vojvodina. **Material and Methods.** Using a descriptive, epidemiological study we analysed the occupational anthropozoonoses occurring in the Vojvodina which were registered at the Institute of Occupational Health Novi Sad, Serbia, from 1992 to 2016. **Results.** In the observed period, a total of 481 occupational diseases were identified, out of which 62 (12.9%) cases were the occupational infectious diseases. A total of 19 cases of occupational anthropozoonoses were registered. The average annual incidence of occupational anthropozoonoses was 0.96 per 100,000 employees. The linear trend of annual incidence was negative. The average age was 44.05±8.34 years. Male patients accounted for 73.7% of cases. The average length of service among patients was 19.63±8.2 years. **Conclusion.** In addition to the implementation of available specific immunization measures and personal protection measures that prevent contact with pathogens, it is important to continuously educate workers concerning the workplace risks and perform preventive medical examinations in order to enable early recognition and treatment of the disease. In addition, continuous education of medical practitioners of different specialities would help to better identify occupational anthropozoonoses.

Key words: Epidemiology; Occupational Diseases; Zoonoses; Risk Factors; Epidemiologic Studies; Incidence; Vaccination

Sažetak

Uvod. Antropozoonoze su oboljenja ljudi i životinja koja se pod prirodnim uslovima mogu preneti sa životinje na ljude. Cilj rada je analiza karakteristika i vrsta profesionalnih štetnosti koja dovode do profesionalnih antropozoonoza i njihove učestalosti u privrednim delatnostima Autonomne Pokrajine Vojvodine.**Materijal i metode.** Upotrebom deskriptivne epidemiološke studije, izvršena je analiza profesionalnih antropozoonoza u Vojvodini u periodu od 1992. do 2016. godine koje su registrovane u Zavodu za zdravstvenu zaštitu radnika Novi Sad, Srbija. **Rezultati.** U posmatranom periodu utvrđeno je ukupno 481 profesionalno oboljenje, od kojih su 62 slučaja (12,9%) profesionalna infektivna oboljenja. Registrovano je 19 slučajeva profesionalnih antropozoonoza. Prosečna godišnja stopa incidencije iznosila je 0,96 profesionalnih antropozoonoza na 100 000 zaposlenih. Linearni trend kretanja prosečne godišnje incidencije bio je negativan. Muškarci su činili 73,7% slučajeva. Prosečan uzrast obolelih bio je 44,05 ± 8,34 godine. Prosečan radni staž obolelih iznosio je 19,63 ± 8,2 godine. **Zaključak.** Pored primene specifičnih mera imunizacije, ukoliko postoje, kao i ličnih mera zaštite koje sprečavaju kontakt sa uzročnicima, značajna je edukacija radnika o rizicima i sprovođenje preventivnih lekarskih pregleda radi rane identifikacije obolelih radnika. Kontinuirana edukacija lekara različitih specijalnosti pomogla bi češćoj identifikaciji profesionalnih antropozoonoza.

Ključne reči: epidemiologija; profesionalna oboljenja; zoonoze; faktori rizika; epidemiološke studije; incidenca; vakcinacija

Introduction

According to the definition of the Law on Pension and Disability Insurance, occupational diseases are caused by a prolonged and direct impact of the work process and conditions in the workplace, i.e. work tasks that the insured person has performed. The basic requirement for a disease to be acknowledged as occupational is the existence of an objectively proven causal relationship between work done in the workplace and the development of the disease and that the disease is listed in the Regulations for Determining Occupational Diseases (hereafter Regulations) [1, 2].

The current Regulations of the Republic of Serbia, issued in 2003, identify 56 diseases or groups of diseases resulting from exposure to occupational hazards at the workplace. The same Regulations define the types of risks under which diseases occur, as well as more detailed requirements for their acknowledgement [2–4].

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Abbreviations
OID – occupational infectious diseases
APV – Autonomous Province of Vojvodina

The institutions authorized for the acknowledgement of occupational diseases in the territory of the Republic of Serbia are the Institute for Occupational Health of Serbia "Dr. Dragomir Karajović" Belgrade, the Institute for Occupational Health Novi Sad, the Institute for Occupational Health Niš, and the Military Medical Academy Institute for Occupational Medicine Belgrade, which all function in accordance with the current Regulations [5].

According to the current Regulations, occupational infectious diseases (OID) are divided into five groups. They occur as a result of exposure to biological pathogens in the working environment and can be: tropical and imported diseases, anthropozoonoses, viral hepatitides, human immunodeficiency virus infection (and acquired immunodeficiency syndrom as a terminal stage) and tuberculosis. In all of these diseases, it is necessary to prove a contact with the biological agent, as well as spatial and temporal association with the onset of the disease [2]. Tropical and imported contagious diseases occur in workers employed in areas where these diseases are endemic and/or epidemic (viral hemorrhagic fevers, yellow fever, cholera, plague, malaria, leishmaniasis, amoebiasis, etc.) [6].

There are several basic ways of acquiring occupational anthropozoonoses:

1. Contact with diseased animals, their corpses, and infected products of animal or plant origin (skin, hair, wool, buns, meat, fur, pettitoes, milk, grain, straw, hay, cotton).
2. Contact with a vector, i.e. a living organism that transmits an infectious agent between people or between animals and humans.
3. Working under conditions leading to a contact with infected soil, water and air [7–9].

Anthropozoonoses are diseases of humans and animals, which can be transmitted from animals to humans under natural conditions. They often occur in a particular geographical area and are most commonly seasonal, sporadic or in the form of minor epidemics [10–12].

Anthropozoonoses are as old as human race and accompany it through history. They have epidemiological-medical and social significance [13]. By 2001, around 1,415 pathogenic agents infectious for humans were identified, of which 217 viruses and prions, 538 bacteria and rickettsia, 307 fungi, 66 protozoa, and 287 worms. Out of these agents, anthropozoones account for 61% (about 868 species) [14].

People working with diseased animals or their products can be infected with various agents from the group of anthropozoonoses [15]. Lack of laboratory diagnostics prevents the perception of the extent and actual frequency of these diseases, which most often go underrecognized, and consequently underreported [16].

In the territory of the Autonomous Province of Vojvodina (APV), the following anthropozooneses have been registered so far: Queensland fever (Q fever); Lyme borreliosis; leptospiroses; tularemia or rodent plague; brucellosis, and anthrax. In addition to these anthropozoonoses, viral hemorrhagic fevers have been recorded in the territory of Serbia.

The aim of our study was to analyze characteristics and types of occupational hazards that may lead to occupational anthropozooneses, determine their incidence in the economic activities of the APV, as well as to propose adequate prevention measures.

Material and Methods

Research was designed as a descriptive epidemiological study. The research materials were documentation on occupational anthropozoonses recognized in the APV during a twenty-five-year period (from 1992 to 2016).

The source of data for this study were expert reports about occupational diseases in the APV, established at the Novi Sad Institute of Occupational Health, the only authorized institution for acknowledging occupational diseases in the APV territory, in accordance with the current Regulations [2–5].

Except of demographic characteristics, the expert reports contain data about work history, epidemiological information, performed laboratory procedures, and reports of other specialists who estimated the working ability.

The common requirements for the acknowledgement of occupational contagious diseases are:

– The employee worked on jobs and work places where contact was made with the causative biological factor, i.e. there is evidence of temporal and spatial association;
– There is a clinical picture of an infectious disease in an acute, subacute and chronic stage, or of a condition after recovery from the disease (the diagnosis established by an infectious disease specialist);
– There is data providing information on the current epidemiological conditions in the family and in the environment - outside the workplace.

In addition to the above-mentioned requirements, for tropical imported diseases, it is necessary to determine whether the employee has been working in areas where these diseases are endemic and/or epidemic. For the acknowledgement of viral hepatitides and HIV infection (AIDS) as occupational diseases, it is necessary that there was a proven parenteral contact of the patient with a biological agent. According to the Regulations, the clinical picture of tuberculosis should be caused by tuberculosis bacilli resistant to anti-tuberculosis drugs [2–4, 15].

Data on employees classified by economic activities were taken from the statistical annals of the former Federal Republic of Yugoslavia, the former Federal Republic of Serbia and Montenegro, and the current Republic of Serbia, as well as from the database of the National Institute for Statistics.

Characteristics of the studied groups were presented using basic indicators of descriptive statistics. The incidence of occupational diseases and occupational infectious diseases was calculated per 100,000 employed people.

Distribution of characteristics by the groups was analysed by the non-parametric chi-square test, or by Fischer test in cases where any of the studied groups had fewer than five elements. For $p<0.05$ the difference between the observed distributions was considered statistically significant, and $p<0.01$ indicated a highly statistically significant difference.

Comparison of average values for age and length of service between the two groups was done using the t-test. Mean values for more than two groups which simultaneously appear were compared with the ANOVA Tucky's test. All calculations were made with the statistical software Statistica 12 [17].

The study was carried out at the Institute of Occupational Health Novi Sad, which is a teaching base of the University of Novi Sad, Faculty of Medicine Novi Sad, Serbia.

Results

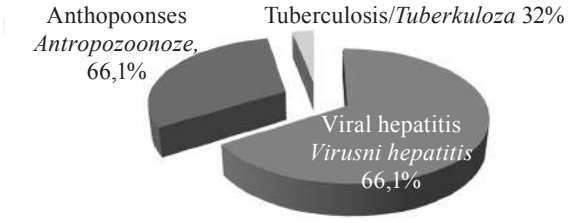
In the study period, a total of 481 occupational diseases were registered in APV, of which 62 cases of OID. Out of 62 cases of OID, there were 41 cases of occupational viral hepatitides, 19 cases of occupational anthropozoonoses, and two cases of occupational tuberculosis **(Graph 1)**.

Of 19 cases of occupational anthropozoonoses, a total of 11 cases of Q fever were registered, and they accounted for 57.9% **(Graph 2)**.

As shown in **Graph 3,** anthropozoonoses mainly sporadically occurred, except an epidemic of Q fever in 2001 (in the period from May 23 – June 6, 2011). There were 10 registered cases in the epidemic and all employees worked in the meat industry, on the cattle slaughter line, nine workers and a supervising technologist. In addition to this epidemic, in the period from 1992 to 2011 there was a sporadic case of occupational Q fever in 2006, in a person who worked in pig breeding. Of all anthropozoonosis cases, five were female and all had Q fever. Average of 0.76 cases of anthropozoonoses per year were registered in the observed period. The average incidence was 0.96 per 100.000 employees in the fields of agriculture, agricultural services, hunting, fishing, forestry and food industry.

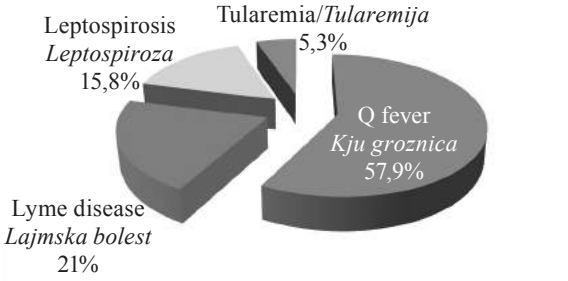
The linear trend of average annual incidence was negative with a decrease coefficient of -0.002 per year, described by the equation $y = -0.002x + 0.560$ **(Graph 4)**.

Male patients accounted for 73.7% of cases but there was no statistically significant difference by gender in groups of causative agents (0.1540) **(Table 1)**. As regards age of patients, there was no statistically significant difference between those with occupational anthropozoonoses ($p = 0.4919$). The oldest average age was recorded in workers with Lyme disease (48 years), and the youngest was a patient with Q fever (41.8 years). Fischer's nonparametric test confirmed that there was no statistically significant difference in the distribution of diseases with regard to education level ($p=0.5952$). Tularemia was found in one worker aged 53, with a 15-year length of service and 2 years of exposed work.



Graph 1. Distribution of occupational infectious diseases by causative agents, 1992–2016 in the Autonomous Province of Vojvodina

Grafikon 1. *Struktura profesionalnih infektivnih oboljenja prema uzročniku bolesti u Vojvodini, od 1992 do 2016. godine*



Graph 2. Distribution of occupational anthropozoonoses by causative agents in the Autonomous Province of Vojvodina, 1992–2016

Grafikon 2. *Struktura profesionalnih antropozoonoza prema uzročniku bolesti u Vojvodini od 1992 do 2016. godine*

The longest overall length of service was recorded in workers with Lyme disease (27.25 years), and with regard to exposed work in those with Q fever (12 years) **(Table 2)**. Using the ANOVA there were no statistically significant differences between average values of the total ($p = 0.0732$) or the exposed ($p = 0.6387$) length of service. Q fever was the cause of occupational disease in workers employed at the following workplaces: meat production technologist, slaughtering line worker (82% of cases) and pig feeding manager.

Discussion

In the observed period, a total of 19 cases of occupational anthropozoonoses were registered in the APV; Q fever (57.9%), Lyme disease (21%), leptospirosis (15.8%), and tularemia (5.3%). Anthropozoonoses have different etiologies and a polymorphic clinical picture; therefore, without laboratory tests, it is difficult to establish the diagnosis. During our study, occupational anthropozoonoses occurred sporadically and only once as an epidemic. The seasonal character is conditioned by the presence of a vector and reservoir of causative agents, i.e., temporal and spatial contact of people in natural focuses of infection [10, 11].

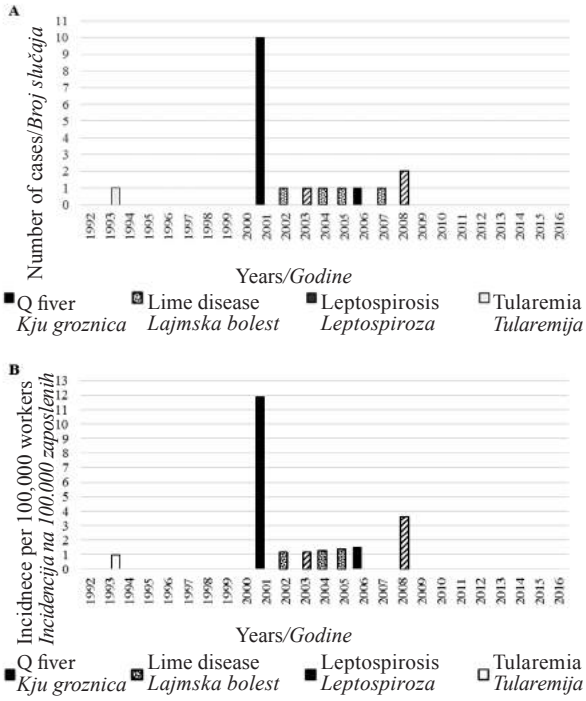
Zoonoses accounted for 30.6% of all OID in our study, which is almost three times higher than in Croatia

Table 1. Analysis of occupational anthropozoonoses in the APV by gender, age and education, 1992–2016
Tabela 1. *Analiza profesionalnih antropozoonoza u Vojvodini prema polu, starosti i stručnoj spremi obolelih u periodu 1992–2016. godine*

Occupational anthropozoonoses *Profesionalne antropozoonoze*		Total (%) *Ukupno (%)*	Q fever *Kju groznica*	Lyme disease *Lajmska bolest*	Leptospirosis *Leptospiroza*	Tularemia *Tularemija*	p
Total/*Ukupno*		19 (100)	11	4	3	1	
Gender/*Pol*	Male/*Muškarci*	14 (73.7)	6	4	3	1	0.1540
	Female/*Žene*	5 (26.3)	5	0	0	0	
Age/*Uzrast*	Mean (X) *Srednja vrednost*	44.05	41.82[a]	48.0[a]	44.0[a]	53	0.4919
	±SD	8.34	8.33	8.83	7.94		
Educational level *Stručna sprema*	8yrs *Osnovna školska sprema*	6	5	0	1	0	
	11-12yrs *Srednja stručna sprema*	12	5	4	2	1	0.5952
	≥16yrs/*Visoka i viša stručna sprema*	1	1	0	0	0	

[a] superscripts mark classes (obtained by the ANOVA) with mean values, *only one patient had tularemia and was not included into the ANOVA

*[a]malim slovima su obeležene klase (dobijene ANOVA analizom) kojima pripadaju srednje vrednosti, *od tularmije je oboleo samo jedan radnik pa nije uvršten u ANOVA analizu*

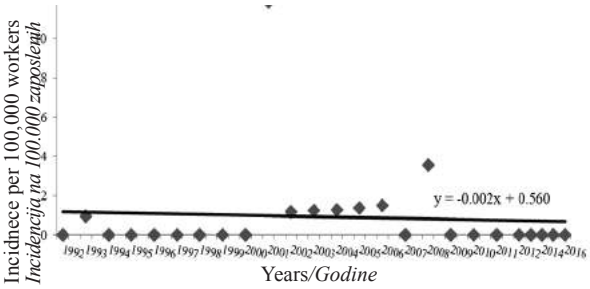


Graph 3. Distribution of occupational anthropozoonoses among workers in agriculture, agricultural services, hunting, fishing, forestry and food industry: A) Number of cases, and B) Incidence per 100.000 workers
Grafikon 3. *Struktura profesionalnih antropozoonoza zaposlenih u poljoprivredi, poljoprivrednoj uslužnoj delatnosti, lovu, ribolovu, šumarstvu i prehrambenoj industriji: A) Broj slučajeva i B) Incidencija na 100.000 zaposlenih*

in the period 2001–2010, as recorded by Krišt D. et al., where they accounted for 10% of all OID (110 OID in total) [18]. An average incidence of anthropozoonoses in Vojvodina in the period 2005–2014 was 7.64 per 100,000 inhabitants; it was the highest in 2005 (18.6 per 100,000) and the lowest in 2010 (3.7 per 100,000) [16]. However, unlike the general population, the average incidence of anthropozooses among workers in our study was six times lower (0.96 per 100,000 workers).

Among occupational anthropozoonoses, males were significantly more frequently affected (73.6%), which is in agreement with the gender distribution of anthropozoonoses among the general population in the areas of the APV, Serbia and the city of Belgrade [19–22].

We identified an epidemic of Q fever in 2001 in a livestock slaughterhouse. There were 10 recorded cases during the epidemic and all affected persons worked on slaughter lines; nine workers and one supervising



Graph 4. Linear trend of the average annual incidence of occupational anthropozoonoses in the Autonomous Province of Vojvodina, 1992–2016
Grafikon 4. *Kretanje linearnog trenda prosečne godišnje incidencije profesionalnih antropozoonoza u Vojvodini od 1992 do 2016. godine*

Table 2. Analysis of occupational anthropozoonoses by total and exposed length of service and workplace in the APV, 1992–2016
Tabela 2. *Analiza profesionalnih antropozoonoza prema ukupnom i izloženom radnom stažu i radnom mestu u Vojvodini od 1992. do 2016. godine*

Occupational anthropozoonoses *Profesionalne antropozoonoze*		Total *Ukupno*	Q fever *Kju groznica*	Lyme disease *Lajmska bolest*	leptospirosis *Leptospiroza*	Tularemia *Tularemija*	p
Total/*Ukupno*		19 (100)	11	4	3	1	
Total length of service/*Ukupan radni staž*	Mean (X) *Srednja vrednost*	19.63	16.36[a]	27.25[a]	23.0[a]	15	0.0732
	±SD	8.2	6.39	8.73	9.16		
Exposed work *Ekspozicioni radni staž*	mean (X) *Srednja vrednost*	10.78	12.0[a]	11.0[a]	9.0[a]	2	0.6387
	±SD	5.02	10.56	5.2	6.0		
Workplace *Radno mesto*	Technologist of meat production/*Tehnolog procesa proizvodnje mesa*	1 (5.3)	1	0	0	0	
	Slaughtering line worker/*Radnik na liniji klanja*	9 (47.4)	9	0	0	0	
	Pig feeding manager *Poslovođa tova svinja*	1 (5.3)	1	0	0	0	
	Game warden *Lovočuvar*	1 (5.3)	0	1	0	0	
	Watering electrician *Električar zalivnog sistema*	1 (5.3)	0	1	0	0	
	Livestock admin. *Referent stočarstva*	1 (5.3)	0	1	0	0	
	Water plant manager *Rukovodilac postrojenja izvorišta*	1 (5.3)	0	1	0	0	
	Fisherman/*Ribar*	2 (10.5)	0	0	2	0	
	Water pump keeper *Radnik na obezbeđenju vodene pumpe*	1 (5.3)	0	0	1	0	
	Game storehouse keeper/*Magacioner divljači*	1 (5.3)	0	0	0	1	

[a] superscripts mark classes (obtained by the ANOVA) with mean values
[a] *malim slovima su obeležene klase (dobijene ANOVA analizom) kojima pripadaju srednje vrednosti*

technologist. In addition to these cases, another case of occupational Q fever was reported in 2006 in a person who worked on pig breeding. In our study, most cases of this disease were registered in late May and early June (90% of cases of occupational Q fever). In Vojvodina, about 90% of cases of Q fever in the general population were recorded at the end of winter and in early spring, because the main source of the infection with Coxiella burnetii (C. burnetii) are domestic animals especially sheep in the season of bringing forth lambs. Human Q fever is mainly transmitted by aerosol infection. Occurrence at a certain time of the year is a consequence of the presence of the transmitter and the reservoir of the causative agent [10, 11, 20].

Unlike Vojvodina, where occupational Q fever was the leading zoonosis, with the prevalence of 57.9%, in Croatia in the period 2001–2010, out of the registered 11 cases, hemorrhagic fever was the most common (54.5%, 6 cases), followed by Q fever (45.5%, 5 cases). All persons affected with Q fever were working with cattle - 4 graduated veterinarians and one cattle breeder. Other occupational anthropozoonoses were not registered in Croatia in the mentioned period [18].

Q fever was first described in the Balkans in 1941 and 1942 as "Balkan Influenza" and since then it has been reported in smaller and larger epidemics and epizooties. The most frequent cause of their occurrence is uncontrolled movements of livestock, mainly of sheep and goats. One of the main features is an aerogenous pathway of transmission that is not specific to other anthropozoonoses [11, 23].

Q fever is endemic in Vojvodina compared to other parts of Serbia. The main reason for endemo-epidemic maintenance of Q fever was in the fact that the nomadic herds of sheep arrived from the western regions of former Yugoslavia to Vojvodina, particu-

larly in winter months of the year. In a large number of cases it manifests with a wide spectrum of symptoms, so it remains unrecognized and is often defined as an unspecified febrile condition [19, 23].

An analysis of C. burnetii seropositive persons showed that 9.3% of Vojvodina's population was in contact with the bacterium, and it was the most prevalent in Northern Banat (24.1%) [20]. An Italian study conducted among cattle breeders showed that 84% were seropositive to Q fever [24].

The prevalence of Q fever among OID in our study was 58%, while in the period 1997–2001 it accounted for 7.4% of all anthropozoonoses in the general population. The average age of Q fever patients in our study was 41.8 years, and in the general population the highest incidence was recorded among the population aged 40–49 years [21].

Lyme disease, in the twenty-five-year period of our study, accounted for 21% of anthropozoonoses, preceded by Q fever (58%); four cases of definite occupational diseases were confirmed, and all of them were males.

Since 1990, Lyme disease occurred in an upward linear trend until 2009, when the highest incidence among the general population to date was recorded (14.5/100.000). From 2009 to 2014, the incidence was steadily declining and in 2014 it was 5.6 per 100.000 inhabitants in Vojvodina [25]. According to a study by Nađ Čik E. from 2003, the incidence of Lyme disease in the general population was steadily increasing from 1988 to 2001, reaching its peak with 9.9 per 100.000 inhabitants of Vojvodina (the average in the period was 4.83/100.000), and the gender distribution in the general population was almost the same. Unlike the general population, acknowledged occupational cases of Lyme disease were recorded only sporadically, although it accounted for 97% of all vector-borne diseases [26, 27]. The control of Lyme disease was ongoing since 1990; however, according to the current Law on Protection from Contagious Disease from 2016, this disease is no longer under necessarily epidemiological reporting by physicians [28].

In the period 2000–2007, Bilski B. registered 218 cases of acknowledged occupational Lyme disease in the province of Wielkopolska in Poland, which has a two times larger population than Vojvodina with only 4 registered cases. All the cases were from the same economic sectors as in Vojvodina, i.e. agriculture, hunting, fishing and forestry, with 89% of males [29]. Lewandowska A. investigated occupational Lyme disease among forestry inspection workers and came to the conclusion that Lyme disease was more common in older workers, over 45 years of age [30]. The average age in our study was 48 years.

Although Lyme disease is vaccine preventable disease, there are still other prevailing preventive measures against the disease, including self-examination after staying at places where tick contact is expected, wearing protective clothing, avoiding areas with ticks, if possible, and the use of repellents [31, 32].

Occupational leptospiroses in APV accounted for 16% of all established occupational anthropozoonoses in the period 1992–2016. In the general population of APV in the period 1997–2001, an average of 17.2 cases were reported annually (5.6% of all anthropozoonoses), while in the period 2002–2007, the annual average was 16.2 cases and the majority were males (95%) [12, 21]. In our study, among occupational leptospiroses all three cases were men who were in contact with contaminated water. The studies by Šeguljev Z. and Petrović M. showed that in two thirds of leptospirosis cases in the general population contamination occurred through contact with water [20, 21], while in Šeguljev's study from 1994, 5.3% of all diagnosed leptospiroses in the population of Vojvodina occurred due to working in water [33]. Petrović M. et al. stated that the working population was the most vulnerable for leptospirosis infection, with men being nine times more frequently affected than women (1.56 vs. 0.17 per 100.000 inhabitants) [21]. Considering that no professional leptospirosis was registered in our study before 2003, it is likely that they were not recognized as occupational diseases.

In the period 2000–2009 in Vojvodina a total of 142 cases of leptospirosis infection among the general population were reported, 17 of which had fatal outcome. The high mortality rate of 11.9% is explained by the recording of only the most severe cases in which the fatal outcome is much more frequent [34].

We found only one case of occupational tularemia in Vojvodina. According to the results of the study released by Ljubić B. (between 1988 and 2007) there were total of 15 cases of tularemia, which accounted for 0.07% of all anthropozoonoses in the general population in the area of the city of Belgrade [10].

In Poland, in the period 1996–2009, there were 4 to 6 cases of tularemia annually, with an incidence of 0.01 to 0.02 per 100.000 inhabitants. In a study conducted by Żukiewicz-Sobczak W. in 2014 among forest workers, 3.2% of tested workers were seropositive to tularemia [35].

Prevention of anthropozoonoses includes primarily veterinary measures aimed at suppression of anthropozoonoses in animals and zoohygienic measures preventing transmission of anthropozoonoses to humans: adequate keeping of animals in appropriate spaces outside human settlements; application of safety gears: clothing, footwear, gloves, mask, protective goggles; measures of disinfection, disinfestations and pest control in the premises where animals are kept, and occasionally also in settlements or popular outdoor locations; prohibition of movement of livestock and their grazing through and in the vicinity of the settlement as well as in areas for rest and recreation of people; prohibition of the use of products and raw materials from dead animals; correct removal of animal waste, corpses and manure; continuous health education at all levels (experts, population and especially professionally exposed subjects). Zoohygienic measures are simple, easily applicable and relatively inexpensive [21, 36].

With some anthropozoonoses there is the possibility of applying specific and effective prevention measures such as vaccination. Prior to vaccination against Q fever, the serological status of a person must be

checked for the presence of antibodies against Q fever. This is done with the Q fever skin test, in case the vaccination is contraindicated if the person has been vaccinated or already had Q fever infection [37]. Vaccination against Q fever decreases its occurrence among workers who are professionally at risk. However, in Australia, in the period 2000–2006, 8% of vaccinated persons still developed signs of disease after occupational exposure [38].

Vaccination against Lyme disease is carried out with a recombinant vaccine. It contains protein A (rOspA) of Borrelia burgdorferi. The protein is obtained by expressing the gene through Escherichia coli and then is purified [31].

Staff training is also one of the ways of preventive action. In Serbia, the training of employees, as well as those exposed to infectious agents, is regulated by the Law on Safety and Health at Work (Art. 27–31 of the Law). Training of employees for safe and healthy work is performed by the employer both theoretically and practically, in accordance with the education program adopted by the employer. Assessment of the theoretical and practical skills of an employee is done at the workplace. Periodic assessments of employees working at an increased risk workplace are carried out no later than one year after the date of the previous assessment, and at other workplaces no later than four years after the previous assessment [39].

Conclusion

After 2008, we did not register cases of occupational anthropozoonoses in Vojvodina. In addition to the reduced economic activity in the country, another explanation for this may also be that professional knowledge about zoonosis among primary care physicians in Vojvodina is not at a satisfactory level. Therefore, it is necessary to carry out continuous education.

There are several medical, social and economic consequences involved in the development of occupational anthropozoonosis. These include the loss of a trained and skilled worker for a certain period of time or permanently, depending on the type of infection and the possibility of healing and further transmission of the infection that can have serious and sometimes fatal consequences for both healthcare or other economic sector workers, as well as for patients. Applying effective control measures, identifying risks and hazards, risk prevention when possible, appropriately timed medical examinations of workers at an increased risk and treatment of recognized cases are essential for further reduction of the number of occupational infections among exposed workers.

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SUICIDE USING A SIMPLE CRUDE HOMEMADE FIREARM (ZIP GUN) – A CASE REPORT

SAMOUBISTVO IZVRŠENO KORIŠĆENJEM JEDNOSTAVNOG VATRENOG ORUŽJA IZRAĐENOG U KUĆNIM USLOVIMA – PRIKAZ SLUČAJA

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Summary

Introduction. For homemade firearm, as well as for blank pistol, tear gas gun or cap pistol, that are adapted to use as firearm, there is a commonly used term - zip gun. Presented zip gun is one of the simplest crude homemade firearms that was found among reviewed articles. **Case report.** We present a case where a young man committed suicide by using a very simple, crude zip gun. The iron tube was used as a barrel. At one end of the iron tube a hunting cartridge was inserted. That end of the tube was closed by iron cylinder with a screw breaching through the center. The decedent was holding the metal tube i.e. the barrel in left hand. He was holding a hammer with his right hand and trying to hit the head of the screw which was, in this case, used as a firing pin. **Conclusion**. Authors would like to emphasize the importance of thorough investigation and detailed documentation of the crime scene. Particular attention is needed in cases were unusual metal or other parts can be found at the scene from witch a crude homemade gun can be made. We also emphasize that, because of the low power of these kind of firearms and possibility of surviving the shot, if the vital structures are not damaged, an urgent and adequate medical intervention could save the injured persons life.

Key words: Suicide; Firearms; Wounds, Gunshot; Forensic Ballistics; Forensic Pathology; Autopsy; Asphyxia; Hemorrhage

Sažetak

Uvod. Za jednostavno vatreno oružje izrađeno u kućnim uslovima, pištolj-plašljivac, gasni pištolj i pištolj na kapisle, koji su modifikovani tako da mogu da ispaljuju pravi projektil, postoji zajednički naziv zip-gun. Oružje opisano u ovom radu, predstavlja jedno od najjednostavnijih vatrenih oružja, izrađenih u kućnim uslovima, koje se moglo pronaći među objavljenim stručnim, naučnim radovima. **Prikaz slučaja.** Prikazan je slučaj samoubistva mlađe muške osobe, izvršenog pomoću vatrenog oružja vrlo jednostavnog i prostog mehanizma i izrade. U ovom slučaju, gvozdena cev je korišćena kao cev oružja. U jedan kraj cevi ubačena je lovačka patrona, a kraj cevi je zatvoren poklopcem sa metalnom pločicom, kroz čiju sredinu je probijen šraf. Pokojni je držao cev u levoj ruci, a čekić u desnoj ruci, i pokušavao je čekićem da udari glavu šrafa, koji je u ovom slučaju imao ulogu udarne igle. **Zaključak.** Prikazom ovog slučaja autori bi želeli da naglase važnost detaljne istrage i dokumentovanja predmeta na samom licu mesta. Posebnu pažnju potrebno je obratiti u slučajevima u kojima se na licu mesta nalaze neobični delovi metala ili drugog materijala, a koji bi mogli biti upotrebljeni za izradu prostih tipova vatrenog oružja. Takođe naglašavamo da, zbog niske snage ovih jednostavnih oružja, kao i realne mogućnosti preživljavanja nastale strelne rane, ukoliko vitalne strukture nisu oštećene, hitna i adekvatna medicinska intervencija može spasiti život povređenoj osobi.

Ključne reči: samoubistvo; vatreno oružje; rane od oružja; forenzička balistika; forenzička patologija; autopsija; asfiksija; krvarenje

Introduction

For homemade firearm, as well as for blank pistol, tear gas gun or cap pistol that are adapted to use as firearm, there is a commonly used term - "zip gun"[1, 2]. Zip guns were most popular in 1950s and 1960s, predominantly in urban city areas, where they were used by young gang members in warfare. Most of the zip guns were very simple and crude in construction, made of block of wood, a car antenna was used as the barrel, a nail as the firing pin, and rubber bands were used to propel the pin [3]. Because of crude construction the quality of these weapons was low. They could fire projectiles at low velocity, had limited accuracy and commonly misfired [4]. It was not uncommon that the firer of such weapon goes worse than the intended victim. Nowadays, zip guns are replaced with well manufactured, commercially made firearms that are relatively easily available. Crude homemade firearms are likely to be found in developing countries where either the commercial firearms are still too expensive for poor population, or getting licensed firearm is difficult because of very stringent licensing policy [5]. We present a case of suicide which was committed with a zip gun. Presented zip gun is one of the simplest crude homemade firearms that were found among reviewed articles of similar theme.

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Figure 1. A 20–30 year old male found dead in an armchair, lots of small junk scattered around the room
Slika 1. *Muškarac starosti 20–30 godina pronađen mrtav u fotelji, mnoštvo sitnog smeća razbacanog okolo po sobi*

Figure 3. Wooden hammer that has a thick metal plate with many small cone-shaped wedges
Slika 3. *Drveni čekić sa metalnom pločom na kojoj se nalazi mnoštvo klinastih ispupčenja*

Case report

A 20-30 year old Caucasian male was found dead in his apartment, recumbent backwards in an armchair. His head was resting on his chest. The chest was covered with dry blood that originated from both, the nose and the mouth. There were two wounds seen on the upper lip. His left arm was above the right one, lying on his lower abdomen and there was soot found on the left hand. Fingers were bent as he was holding something in his hand just before death. The right arm was below the left one, with his hand resting on the right thigh. On the right hand no soot was found. The body was transported in order to perform an autopsy.

The investigation was thorough and every detail that could lead to solving this case was photographed and taken into account. The residence was untidy with lots of small junk scattered around the room where the deceased was found **(Figure 1)**. Something brought to investigators' attention. There was an iron tube lying on the floor in front of the armchair **(Figure 2)**. The tube was approximately 22 cm long, about 3 cm in diameter, and was open at one end. At the other end there was hunting cartridge inserted. The cartridge was labeled *CHEDITE 12* and at least three scorings were found on the percussion cap, two of which were on the periphery. Close to the armchair a wooden hammer was found **(Figure 3)**. On the striking part of the hammer there was a thick metal plate with many small cone-shaped wedges. On the table that was in front of the armchair, a small cylinder (4.5 x 4.5 cm) was found. At one end the cylinder was closed with a rectangular plate, which was welded for the cylinder edges and had somewhat greater dimensions than the cylinder. In the middle of the plate a screw was breaching through ending with a spike inside the cylinder **(Figure 4)**.

During autopsy it was noted that the deceased was 183 cm in length, well nourished, with well-developed muscular build. There was a stellate-shaped gunshot wound across the lips, 3 cm in diameter with searing of the wound edges and soot present inside the mouth. Double fractures of the

Figure 2. Iron tube lying on the floor, approximately 22 cm long, about 3 cm in diameter, with hunting cartridge inserted
Slika 2. *Gvozdena cev na podu, dužine oko 22 cm, prečnika oko 3 cm, sa ubačenom lovačkom patronom*

Figure 4. Small cylinder closed with a rectangular plate, screw breaching through the plate and ending with a spike used as a firing pin
Slika 4. *Poklopac sa metalnom pločicom, kroz koju je probijen šraf čiji vrh je služio kao udarna igla*

maxilla and mandible were found as well as round-shaped defect of the tongue and hard palate. On the base of the tongue, two slightly deformed pellets were found. The wound course involved base of the skull with a narrow hole in the skull, about 2 x 0,3 cm in size, just on the right side of sella turcica. On the right side of the brain stem two slightly deformed pellets were found. In exception to small subarachnoid hemorrhage no damage to the brain was done. There was a lot of blood in the trachea and main bronchi as well as massive blood aspiration. Microscopic findings confirmed massive blood aspiration. Toxicology screening was negative.

After considering autopsy findings, police investigation and all surrounding circumstances the mechanism, cause and manner of death were determined. It was particularly interesting that there were not enough signs of brain damage to proclaim brain injury as a cause of death. So, the mechanism of death was mechanical asphyxia due to massive blood aspiration and the manner of death was determined as suicide.

Discussion

Popularity of crude homemade firearms past a few decades ago. Zip guns involved in death cases are rarely to be seen especially in developed countries. There were some cases of crude guns that were reported predominantly from developing countries and countries with low social standard, where commercially made guns are elusive for the majority of the population [5]. Definis Gojanović reported six cases of accidental death using handmade or improvised firearms [6]. It is even more interesting having in mind that there was a war in Croatia not so long ago where all kinds of firearms were easily available and accessible to the population. Cunliffe at al. reported a case of suicide using unusual homemade firearm [4]. In order to make such a firearm one must have some mechanical knowledge as well as access to necessary parts. This case also emphasizes the importance of thorough investigation of the death scene and interviews with witnesses. Homemade firearms are often so crudely constructed that they can be a greater danger to the firer than to the intended victim. Mobus and Eberhardt reported a case of fatal accident due to homemade pistol that was not safe for use [7]. The weapon had fired while reloading killing a person nearby. In accordance with the low power of some crude made zip guns, the damage of vital organs can be avoided and an injured person can have good chance of surviving the shot. Similar case was reported by Alessi et al. [8]. There were some papers among reviewed articles which presented different kinds of zip guns but all of those firearms were somewhat complicated to produce in meaning that some mechanical knowledge and adequate materials were needed [9–11]. Hartwig at al. reported three cases of suicide where decedents were using a homemade firearm with rudimentary triggering mechanisms [12]. In order to fire the shot the percussion cap had to be hit with a hammer or a rock from behind. The last mentioned case report is in someway similar to our case, i.e. the triggering mechanism in both cases involves a hit with a hammer in order to detonate the cartridge percussion cap. Bulent Uner et al. presented an unusual firearm which is usually used against moles but in this case it was used for other purpose [13].

We present a case where a young man committed suicide by using a very simple, crude zip gun, whose parts were previously described. The iron tube was used as a barrel. At one end of the iron tube a hunting cartridge was inserted filled with lead pellets. After insertion that end of the tube was closed by a small iron cylinder with a spike from the inside that was used as a firing pin. The decedent was holding the metal tube i.e. the barrel, in left hand which was above the right hand and had soot on it. He was holding the hammer with his right hand and trying to hit the head of the screw which was in this case used as the firing pin. According to three scorings found on the percussion cap it is possible that the shot misfired for the first two times after being struck with the hammer.

Conclusion

By presenting this case authors would like to emphasize the importance of thorough investigation and detailed documentation of the crime scene. Particular attention is needed in cases where unusual metal or other parts can be found at the scene from which a crude homemade gun can be made. Relatively different gunshot wound can be present on the corpse in accordance with the type of firearm used. We also emphasize that, because of the low power of these kinds of firearms and the possibility of surviving the shot if the vital structures are not damaged, an urgent and adequate medical intervention could save the injured person's life.

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INFLUENCE OF METABOLIC DISORDERS ON PHENOTYPIC MODULATION OF VASCULAR ENDOTHELIUM IN TYPE 2 DIABETES MELLITUS

UTICAJ METABOLIČKIH POREMEĆAJA NA FENOTIPSKU MODULACIJU VASKULARNOG ENDOTELA U TIPU 2 DIJABETESA MELITUS

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Summary

Introduction. Endothelium is a dynamic, strategically positioned defensive regulator of vascular homeostasis. **Physiology and Pathophysiology of Vascular Endothelium.** Endothelial phenotypic modulation involves five basic characteristics: the expression of leukocyte adhesion molecules, the production of cytokines, change in the shape and the permeability of the endothelium, prothrombotic changes and upregulation of autoantigens. **Obesity, Metabolic Inflammation and Vascular Endothelium** One of the most important pathophysiological manifestations of adiposopathy may be the phenotypic conversion of vascular endothelium. **Insulin Resistance and Vascular Endothelium.** Under the conditions of insulin resistance and consequent hyperinsulinemia, there is imbalance between the production of endothelial vasoconstrictors and vasodilators, increased expression of adhesion molecules, and platelet hyperreactivity. **Hyperglycemia and Vascular Endothelium.** Hyperglycemia causes endothelial dysfunction by various mechanisms that involve activation of polyol pathway and production of sorbitol, increased formation of advanced glycation end products, activation of various isoforms of protein kinase C and activation of hexosamine pathway. **Dyslipidemia and vascular endothelium.** Dyslipidemia takes an important role in a cascade of pathophysiological processes that result in endothelial activation and chronic dysfunction. **Conclusion.** Hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, visceral obesity and low-grade inflammation are the main factors responsible for development of endothelial dysfunction in type 2 diabetes mellitus.

Key words: Diabetes Mellitus, Type 2; Endothelium, Vascular; Insulin Resistance; Obesity; Dyslipidemias; Hyperglycemia

Sažetak

Uvod. Endotel je dinamičan, strateški pozicioniran odbrambeni regulator vaskularne homeostaze. **Fiziologija i patofiziologija vaskularnog endotela.** Fenotipska modulacija endotela uključuje pet osnovnih karakteristika: ekspresiju adhezivnih molekula, produkciju citokina, promenu oblika i permeabilnosti endotela, protrombotske promene i ushodnu regulaciju autoantigena. **Gojaznost, metabolička inflamacija i vaskularni endotel**. Jedna od najvažnijih patofizioloških manifestacija adipozopatije može biti fenotipska konverzija vaskularnog endotela. **Insulinska rezistencija i vaskularni endotel.** U uslovima insulinske rezistencije i posledične hiperinsulinemije, dolazi do neravnoteže lučenja vazokonstriktora i vazodilatatora, povećane ekspresije adhezivnih molekula i trombocitne hiperreaktivnosti. **Hiperglikemija i vaskularni endotel.** Hiperglikemija uzrokuje endotelnu disfunkciju različitim mehanizmima koji uključuju aktivaciju poliolskog puta i produkciju sorbitola, povećanu proizvodnju, povećano stvaranje završnih proizvoda glikozilacije, aktivacijom protein kinaze C i aktivacije heksozaminskog puta. **Dislipidemija i vaskularni endotel**. Dislipidemija ima važnu ulogu u kaskadnom nizu patofizioloških procesa koji za rezultat imaju endotelnu aktivaciju i hroničnu disfunkciju. **Zaključak**. Hiperglikemija, hiperinsulinemija, insulinska rezistencija, dislipidemija, visceralni tip gojaznosti i inflamacija su glavni faktori, odgovorni za razvoj endotelne disfunkcije u tipu 2 dijabetesa melitus.

Ključne reči: dijabetes melitus tip 2; vaskularni endotelijum; insulinska rezistencija; gojaznost; dislipidemije; hiperglikemija

Introduction

Type 2 diabetes mellitus (T2DM) as a global health problem is not only a metabolic but also is a vascular disease associated with an increased risk of cardiovascular complications [1]. Long-term hyperglycemia and specific metabolic milieu (i.e. visceral obesity, insulin resistance, hyperinsulinemia, atherogenic dyslipidemia), induce low-grade inflammation, oxidative stress, endothelial dysfunction and platelet hyperactivity, contributing to genesis of hypercoagulable state and consecutive various atherothrombotic complications [2].

Endothelium is a dynamic, defensive regulator of vascular homeostasis. Even though it is a monolayer, healthy endothelium is strategically positioned and is able to respond to wide range of stimuli that regulate vascular tone, blood flow, smooth muscle prolif-

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Abbreviations

T2DM – Type 2 diabetes mellitus
NO – nitric oxide
PGI2 – prostacyclin
ET-1 – endothelin - 1
NF-κB – nuclear factor kappa B
ICAM-1 – intracellular adhesion molecule
VCAM-1 – vascular cell adhesion molecule
vWF – von Willebrand factor
PAI-1 – plasminogen activator inhibitor-1
TF – tissue factor
ROS – reactive oxygen species
DAMP – dammage-associated molecular paterns
NLRP3 – nucleotide-binding domain, leucine-rich repeat, pyrin domain containing 3
TLR4 – toll-like receptor 4
TNF-α – tumor necrosis factor-alpha
IL – interleukin
PI3K – phosphatidylinositol-3 kinase
MAPK – mitogen-activated protein kinase
AGE – advanced glycation end products
PKC – protein kinase C
TAFI – Thrombin activatable fibrinolysis inhibitor
HDL – high-density lipoprotein
sdLDL – small dense low – density lipoprotein
FFA – free fatty acids
VLDL – very low density lipoproteins
oxLDL – oxidised LDL
gLDL – glycated low density lipoprotein
AT – adipose tisue
Lp(a) – lipoprotein-a

eration, cellular adhesion, platelet resistance and vascular inflammation process [3].

Vascular endothelium in terms of deranged metabolic milieu of T2DM loses antiinflammatory, antiadhesive, antiplatelet abilities, expreme leukocyte and platelet adhesion molecules, promotes smooth muscle cell proliferation and migration. Alteration of endothelium is atherosclerosis-prone site and endothelial dysfunction is not only the initiator of atherosclerotic process but also is an amplificator of cascade of events leading to atherosclerotic plaque development [5]. Therefore, endothelial dysfunction is considered to be a ''sentinel'' event in early atherosclerosis development [4].

In this review, we discuss numerous metabolic factors and pathophysiologic processes involved in ethiopathogenesis of endothelial dysfunction in T2DM.

Physiology and Pathophysiology of Vascular Endothelium

Vascular endothelium plays a critical role in the regulation of blood pressure and the optimum blood flow continuously balancing between vasodilation and vasoconstriction, with the synthesis of a vasodilators, nitric oxide (NO) and prostacyclin (PGI_2), and a vasoconstrictors, endothelin - 1 (ET-1) and endothelium - derived hyperpolarizing factor (EDHF) [6]. It is considered that the bioavailability of NO, the most potent vasodilator, is the premise of vascular health [7]. Nitric oxide enables vasodilation, inhibits the proliferation, migration and differentiation of vascular smooth muscle cells to the intima of the blood vessel, stabilizes the inhibitory subunit of nuclear factor kappa B (NF-κB), that maintains this proinflammatory transcription factor in an inactive state, and thereby inhibits the expression of leukocyte adhesion molecules, the production of chemokines and proinflammatory cytokines [6, 8]. Prostacyclin also exhibits vasodilatory, antiplatelet, and cytoprotective action. Under physiological conditions, adhesion molecules responsible for the control of the migration of leukocytes to the vascular endothelium are not expressed on the cell surface [9]. Also, intact vascular endothelium prevents platelet adhesion and balances between the secretion of procoagulant/anti-coagulant and profibrinolitic/antifibrinolytic substances contributing to the equilibrium of coagulation and fibrinolysis [10].

Endothelial dysfunction is characterized by reduced bioavailability of NO that impairs the endothelium-dependent vasodilator capacity of the vessel wall. Considering the protecting abilities of NO, endothelial dysfunction also involves a certain degree of endothelial cell activation that alters the inert endothelial phenotype into proinflammatory, proliferative and procoagulant one [3, 11]. Vascular endothelium in respond to various stimuli (mechanical, biological, chemical, immune, metabolic) expresses phenotypic changes in terms of endothelial activation. Endothelial activation includes de novo gene expression, the synthesis of proinflammatory cytokines and adhesion molecules as well as increased expression of the adhesion molecules like E-selectin, intracellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) and increased permeability of the blood vessel. Endothelial surface becomes prothrombogenic due to reduced production and expression of molecules such as thrombomodulin, heparan sulfate, NO, PGI_2, accompanied by increased von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), tissue factor (TF) and platelet activating factor (PAF). Synthesized proinflammatory cytokines and chemokines amplify the acute phase response and monocytes recruitment to the site of injury [12]. The different stimuli of the endothelial cell surface have a common denominator, a transcription factor, NF-κB. Activation of NF-κB stimulates the transcription of genes responsible for the genesis of phenotypic conversion of endothelial cells [13]. Endothelial phenotypic modulation involves five basic characteristics: the expression of leukocyte adhesion molecules, the production of cytokines, change in the shape and the permeability of the endothelium, prothrombotic changes and upregulation of autoantigens [12]. Interactions of leukocyte and endothelial cell such as capture, rolling, and firm adhesion are series of overlapping synergistic interactions among numerous adhesion molecules result-

ing in an adhesion cascade. These events are precursors of leukocyte extravasation, one of the starting points for the formation and the development of atherosclerotic plaque at the site of endothelial dysfunction [14].

Numerous factors of deranged metabolic milieu like obesity, low-grade inflammation, hyperinsulinemia, insulin resistance, hyperglycemia and dyslipidemia are responsible for endothelial dysfunction in T2DM [15].

Obesity, Metabolic Inflammation and Vascular Endothelium

Obesity represents the most important independent risk factor for T2DM. Also, the risk for developing T2DM increases with the degree of obesity. Overlap of association between T2DM and obesity is beyond epidemiological data, one of the main pathogenetic mechanisms of these diseases is a chronic inflammatory process characterized by the activation of innate and acquired immunity [16]. Adipose tissue (AT) is a dynamic and metabolic highly active endocrine organ involved in the regulation of immunological, metabolic and cardiovascular homeostasis [17]. However, fat accumulation (adiposity) leads to a number of local (adipocyte and AT morphological and functional abnormalities), as well as systemic pathophysiological disorders termed adiposopathy ("adipose-opathy," or "sick fat") [18]. One of the most important pathophysiological manifestations of adiposopathy may be phenotypic conversion of vascular endothelium in both, the microcirculation and the macrocirculation. Thus, the major clinical consequences, arterial hypertension (predominantly-vasoconstrictive vascular phenotype) and accelerated atherosclerosis (predominantly-proinflammatory vascular phenotype), could be present along with metabolic disturbances found in obesity [19].

According to scientific theory (hypothesis), adiposopathy may initially be present in visceral, pericardial, and perivascular fat depots. Morpho-functional abnormalities in AT, adipocyte hypertrophy and organellar dysfunction (especially mitochondrial dysfunction and endoplasmic reticulum (ER) stress), impaired angiogenesis and hypoxia, insufficient adipogenesis, imbalance between apoptosis and adipogenesis, disturbances in the remodeling or degradation of extracellular matrix (ECM), all could lead to activation of numerous immune mechanisms. Also, adiposopathy is associated with imbalance of pro- and anti-inflammatory adipokines, increased production of reactive oxygen species (ROS) and oxidative stress [20].

Like a classic inflammatory response, the one that develops in dysfunctional AT depots represents an attempt to restore and maintain homeostasis. Initiation and the amplification of the inflammatory response by classical mechanisms is named metabolic (meta) inflammation [21]. Potential inducers of these inflammatory responses could be adipocyte and macrophage related signals in response to hypoxia, necrosis, and/or apoptosis [16]. Moreover, current findings highlight the importance of metabolic stressors (glucose, FFA, palmitate, cholesterol crystals, ceramides, etc) as possible inflammatory inducers [22].

Molecular mechanisms underlying the metabolic inflammation in dysfunctional AT are very complex. Activation of the inflammasomes is a key function mediated by the innate immune system. These complexes activate inflammatory protease caspase-1 and induce inflammation in response to molecules that result from cells' damage or death (necrosis/apoptosis), damage-associated molecular patterns (DAMP). Particularly, the inflammasome nucleotide-binding domain, leucine-rich repeat, pyrin domain containing 3 (NLRP3), could recognize certain metabolic stressors. Caspase-1 further regulates the maturation of proinflammatory cytokines interleukin (IL)-1β and IL-18 or pyroptosis (caspase-1-dependent cell death). Activation of NLRP3 inflammasome may also be associated with DAMP stressors, such as extracellular ATP, hyaluron, amyloid-β fibrils and uric acid crystals. Further, potential inducers of this multi-molecular complex could be ROS, potassium and others [23]. In addition, increase in the extracellular concentration of FFA represents an important metabolic stressor, since FFA serve as TLR4 (toll-like receptor 4) ligands. These receptor protein systems, TLR, activate protein kinases, c-jun N-terminal kinases (JNKs). In the human TLR system, TLR 2 and TLR4 have significance in metabolic disorders [24].

The presence (migration) of macrophages in AT present the initial steps of obesity induced metabolic inflammation. Adipose tissue macrophages can be distinguished into M1 and M2 macrophages. In metabolic homeostasis, M2 phenotypic form is predominantly present in visceral AT. In response to perturbation in dysfunctional AT, resident macrophages shift their polarization status. Classically activated or M1 macrophages stimulated by interferon γ (IFN-γ) express pro-inflammatory phenotype and participate in the polarization Th1 adaptive immune response by producing IL 12. The cytokine profile characterizing the M1 phenotype includes tumor necrosis factor-alpha (TNF-α), IL-1β, IL-12, and IL-23. Alternatively activated or M2 macrophages, stimulated by Th2 cytokines, secrete anti-inflammatory cytokines IL-10, IL-1 receptor antagonist (IL-1Ra), transforming growth factor-β (TGF-β) and other factors involved in tissue and fibrous replenishment processes [25].

Signal mechanisms from dysfunctional adipocytes affect the activation and status of local macrophages. Also, chemokines monocyte chemotactic protein-1 (MCP-1) and leukotriene B4 (LTB4) secreted from adipocytes attract monocytes in AT, where they are further differentiated into tissue macrophages. Locally in AT, macrophages form aggregates "crown-like structures" around necrotic adipocytes (necrotic/apoptotic) and residual lipids, and release cytokines, most notably TNF-α. In addition to macrophage, other immune cells have multiple interaction and exhibit their

main effector functions in the process of migration control, activation and polarization of macrophages [21]. Regulatory T lymphocytes CD4 + secretes anti-inflammatory cytokines that inhibit macrophage migration and affect their polarization in M2 phenotype. Cytotoxic CD8 + T lymphocytes infiltrate AT and produce proinflammatory cytokines and activate M1 macrophage phenotype. Also, B lymphocytes further promote activation of T lymphocyte and polarization of macrophage to the proinflammatory M1 phenotype. In addition to mastocyte, resident eosinophils can participate in maintaining M2 polarization status by secreting IL-4 and IL-13. Moreover, local macrophages produce chemokines, which then further promote systemic inflammatory response [26]. The development of obesity-associated systemic inflammation may lead to target organ dysfunction and clinical manifestations of adiposopathy, most notably T2DM [27].

Insulin Resistance and Vascular Endothelium

Under the conditions of insulin resistance and consequent hyperinsulinemia, there is decreased activation of the phosphatidylinositol-3 kinase (PI3K) accompanied by an enhanced activation of mitogen-activated protein kinase (MAPK) signaling pathway, that regulates growth, mitogenesis and differentiation. Using MAPK-dependent signaling pathways, insulin stimulates ET-1 production [28]. Consequence of the reduced activation of PI3K is decreased production of NO accompanied by an increased formation of ROS that induces oxidative stress. Activation of this MAPK-dependent signaling pathway also leads to an up-regulation of PAI-1 and increased expression of adhesion molecules, VCAM-1 and E-selectin. This creates a condition for the expression of vasoconstrictive, proliferative, proatherogenic and prothrombotic endothelial phenotype [29].

Defect in insulin action, caused by insulin resistance, is associated with the changes in platelets function. T2DM is associated with persisted abnormal platelet function proven to be present both *in vitro* and *in vivo*, and characterized with a systemic rather than localized stimulation of platelet activation, as well as continuous rather than episodic alteration [30]. Increased number of platelet aggregates in circulation, increased aggregation of platelets after platelet agonists' addition, increased platelet contractility, and presence of elevated plasma levels of their contents [beta-thromboglobulin, platelet factor 4, thromboxane B_2), demonstrate platelet hyperreactivity in T2DM. Platelets in diabetic patients adhere to vascular endothelium and aggregate more rapidly than in healthy people. The most important reason for this is loss of sensitivity to the normal restraints exercised by PGI_2 and NO, generated by the vascular endothelium [31]. Platelet adhesion occurs at the stage of endothelial dysfunction, before the damage of endothelial structural integrity and is caused by the expression of adhesion molecules on activated endothelial cells, platelets and leukocytes [32].

Hyperglycemia and Vascular Endothelium

Hyperglycemia causes endothelial dysfunction by various mechanisms that involve activation of polyol pathway and production of sorbitol, increased formation of advanced glycation end products (AGE), overexpression of AGE receptors, activation of various isoforms of protein kinase C (PKC) and activation of hexosamine pathway. The common path in which all the previous mechanisms meet and continue to cause vascular damage is the pathway of oxidative stress [33].

In conditions of chronic hyperglycemia due to inability of glucose metabolism in fully aerobic glycolysis, glucose is metabolised by alternative pathways. Activation of the polyol pathway enhances the synthesis of the sorbitol that has toxic, osmotic activity and reduces the concentration of myoinositol. At the same time, due to the oxidation of nicotinamide adenine dinucleotide phosphate (NAD(P)H) and the reduction of nicotinamide adenine dinucleotide (NAD+), redox imbalance is created. It reduces the bioavailability of NO and further enhances the oxidative stress [34].

Nonenzymatic glycosylation of proteins in conditions of chronic hyperglycemia results with increased production of AGE. AGE react with intracellular structures, extracellular matrix and circulating proteins, altering their structure and function [35]. Binding of AGE to AGE receptors on endothelial cells, monocytes, macrophages and smooth muscle cells induces oxidative stress and proinflammatory response [36].

Intracellularly, hyperglycemia increases the synthesis of diacyl-glycerol (DAG) which consequently activates PKC. The consequences of PKC activation are the imbalance between NO/ET-1 ratio, with the consequent predominant vasoconstrictive response and the activation of NF-κB with the increased proinflammatory gene expression [35].

Hyperglycemia stimulates increased production of superoxide anion, O_2^- [37]. O_2^- in turn increases the production of hexosamine and AGE, activates PKC, a polyol pathway and NF-κB and also leads to the production of proinflammatory cytokines (IL-1β, TNF-α), expression of adhesion molecules (E-selectin, ICAM-1, VCAM-1) and ET-1 [38]. It is found that concentration of sE-selectin and vWFAg was significantly higher in patients with T2DM in regard to non-diabetics [39].

Numerous complex mechanisms contribute to the diabetic prothrombotic state, such as: endothelial dysfunction, platelet hyperreactivity, increased coagulation and decreased fibrinolysis [40]. Production of TF increases in the presence of low-grade inflammation, commonly associated with type 2 diabetes. Both glucose and insulin levels are responsible for increment of circulating levels of TF, and it seems that they have an additive effect [41]. In a large term study over 18 years, hemoglobin A1c positively correlated with PAI-1 levels, and nega-

tively with tissue plasminogen activator (t-PA), implicating glycemia in modulating fibrinolytic potential. Thrombin activatable fibrinolysis inhibitor (TAFI) antigen levels, as well as TAFI activity are significantly increased in T2DM. Inverse correlation of TAFI antigen levels and D-dimer was found in these patients supporting the role of TAFI in diabetes-induced inhibition of fibrinolysis [42].

Dyslipidemia and Vascular Endothelium

Dyslipidemia takes an important role in a cascade of pathophysiological processes that result in endothelial activation and chronic dysfunction [43]. In T2DM, dyslipidemia is characterized by an increased level of triglycerides, usually accompanied by high total cholesterol level, low concentration of high-density lipoprotein (HDL) cholesterol and by the presence of small dense low – density lipoprotein (sdLDL) [44]. Hyperglycemia, hyperinsulinemia and insulin resistance are considered to be the main factors responsible for the occurrence of dyslipidemia that is usually expressed phenotypically by the presence of type IV or IIb hyperlipoproteinemia. Long-term hyperglycemia leads to protein glycation, thus resulting with structural and functional disorders of apolipoproteins in different lipoprotein particles, lipoprotein receptors and the enzymes involved in the lipid metabolism [45]. Insulin resistance and subsequent hyperinsulinemia due to reduced insulin sensitivity lead to increased liver synthesis of free fatty acids (FFA), triglycerides and very low density lipoproteins (VLDL), large, triglyceride-rich lipoprotein particles. High affinity of the lipoprotein lipase for chylomicron hydrolysis contributes to slow catabolism and accumulation of VLDL particles as well as chylomicrons and VLDL remnants in blood during postprandial lipemia [46].

In terms of hypertriglyceridemia it increases the activity of the cholesteryl ester transfer protein (CETP), which enables the exchange of triglycerides from the triglyceride-rich lipoproteins to HDL and LDL particles, which in turn give part of their cholesteryl esters to triglyceride-rich lipoproteins. In this way remodeled HDL and LDL particles, now poorer in cholesterol-esters, but richer in triglycerides, become a suitable substrate for hepatic lipase which hydrolyse the triglycerides in these particles, concomitantly creating proatherogenic smaller and denser LDL, sdLDL, also known as sub-populations of LDL-III particles, and the smaller and denser HDL particles [77]. Small dense HDL particles lose their functionality and very rapidly are removed from the circulation, lowering in this way the serum concentration of HDL-cholesterol (especially a protective HDL2 subpopulation) [47].

Due to the structural changes in sdLDL particles, in order to native LDL, sdLDL have a lower binding affinity for LDL receptors, and are predominantly removed from the circulation by binding the scavanger receptors, promoting the formation of foam cells and the development of premature atherosclerosis. The longer retention of sdLDL in subendothelium makes easier their modification by oxidation or the glycation, so their role in the development of endothelial dysfunction is usually attributed to oxidative modification and to the mechanisms of action of oxidised LDL (oxLDL) [47]. Chronic hyperglycemia leads to an increased glycation of LDL particles (glycated LDL, gLDL), with the consequent conformational changes that interfere with their binding to the LDL receptor. Therefore, gLDL stay in circulation for prolonged period of time that may result in oxidation of these particles and the formation of AGE – LDL complex (AGEs-LDL) with pro-inflammatory and pro-atherogenic characteristics. Also, the gLDL have been removed by alternative pathways, independently of LDL receptors. Additionally, gLDL prevents shear stress-mediated L-arginine uptake and NO synthesis and causes increased production of PAI-1 and prostaglandins, while inhibiting the expression of tPA in endothelial cells [46].

In T2DM, there are significant qualitative changes in LDL particles which make them very susceptible to oxidation, especially when they are trapped in intima of blood vessels. In the first stage of oxidation, minimal modifications of LDL particles occur in the form of minimum modified apolipoprotein B, conversion of cholesteryl esters and phospholipids in hydroperoxides, isoprostanes and short length branched-chain aldehydes. These minimally modified LDL particles can stimulate endothelial cells to secrete various chemokines [47].

It is known that oxidatively modified LDL, oxLDL, takes the most important role in the initiation of endothelial dysfunction and the damage of endothelial and smooth muscle cells of the vessel wall. OxLDL binds the released NO, reduces endothelial nitric oxide synthase (eNOS) activity and interferes with the endothelial L-arginine/NO metabolic pathway, thus leading to endothelial vasoconstriction [48]. In the interaction of oxLDL with endothelium, lysophosphatidylcholine is created. Lysophosphatidyl-choline activates protein kinase leading to the formation of superoxide, a reactive oxygen radical, which stimulates further oxidation of LDL particles, as well as it binds NO and thereby inhibits endothelium-dependent vasorelaxation. OxLDL promotes expression of various adhesion molecules (ICAM-1, VCAM-1, E-selectin), activation of NF-κB, increases the expression and production of the ET-1 in endothelial cells and exerts chemotactic effect on monocytes and T lymphocytes. Also, its potent cytotoxic activity and role in apoptosis of endothelial cells has been proven. Binding to scavenger receptors, oxLDL is being taken up by macrophages leading to their activation and transformation into the foam cells which represent pathohistological substrate of early atherosclerotic blood vessels changes [49].

Recent investigations showed that lipoprotein(a) (Lp(a)) as a transporter of oxLDL, particularly in terms of the hyper-Lp(a)-lipoproteinemia, could significantly enhance the effect of oxLDL on the devel-

opment of endothelial dysfunction. Additionally, data suggesting that Lp(a) may contribute to a dysfunctional endothelium in vitro are supported by a number of studies that have demonstrated that elevated plasma Lp(a) concentrations contribute to endothelial dysfunction *in vivo* [50].

Finally, it should be mentioned that a high concentration of FFA caused by excessive influx of FFA from adipose tissue, as well as by impaired uptake by skeletal muscles, participates in the development of endothelial dysfunction in T2DM. Extracellular FFA activate PKC and impair insulin-mediated activation of PI3K thus reducing the bioavailability of NO, the intracellular vasodilator which keeps the endothelium-dependent vasorelaxation by stimulation of guanyl cyclase and increment of intracellular levels of cyclic guanine monophosphate (cGMP) [36]. Additionally, FFA stimulate ROS production and activation of the redox sensitive transcription factors and nuclear receptor systems. Thereby, FFA continue to increase the level of oxidative stress and vascular damage [37].

Conclusion

Hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, visceral obesity and low-grade inflammation are the main factors responsible for the development of endothelial dysfunction in type 2 diabetes mellitus. Altered vascular homeostasis results in decreased bioavailability of nitric oxide, consecutive vasoconstriction, leukocyte adherence, platelet activation, an imbalance between the secretion of procoagulant/anti-coagulant and profibrinolitic/antifibrinolytic substances, increased oxidative stress and vascular inflammation. Morphologically normal arteries with altered functional endothelial response represent a target site of early atherosclerosis. Processes involving endothelial dysfunction promote atherogenesis and atherothrombotic complications at early stage of type 2 diabetes mellitus. Aherothrombosis is the leading cause of morbidity and mortality in patients with diabetes mellitus.

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IN SITU ANALYSIS OF MITOCHONDRIAL RESPIRATORY CAPACITY – FOUNDATION FOR CELLULAR PHYSIOLOGY

IN SITU ANALIZA MITOHONDRIJALNOG RESPIRATORNOG KAPACITETA – OKOSNICA BUDUĆIH ISTRAŽIVANJA IZ ĆELIJSKE FIZIOLOGIJE

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Summary
Mitochondria are ubiquitous organelles of eukaryotic cells and they are the mayor site of generating energy in the form of adenosine triphoshate through the process of oxidative phosphorylation. Analysis and estimation of mitochondrial function is of outmost importance when it comes to studying intracellular energy metabolism, mechanisms of apoptosis, signaling pathways, calcium storage and the pathophysiology of a large spectrum of human diseases including various neurodegenerative diseases, myopathies, metabolic syndromes and cancer. Respiratory capacity analysis covers one of the many roles that mitochondria play in living cells and it provides us with useful data about functional integrity of mitochondria. Assessment of individual respiratory chain complexes or other mitochondrial enzymes has been widely used to estimate mitochondrial function and dysfunction but it neglects the influence of complex structural and functional interplay of mitochondrial proteins and enzymes and plasmic compounds. Another method that emphasises the importance of studying intact mitochondria is *in vitro* technique, and although it has many advantages, in some aspects it is far from being representative when it comes to functional assessment of mitochondria. From the perspective of energy production and consumption, the cardiac muscle is a highly demanding tissue and it is the well functioning of mitochondria that is *conditio sine qua non* for this nature to be fulfilled. In cooperation with the University of Split School of Medicine in Split and under the mentorship of Prof. Marko Ljubkovic, the Department of Physiology of the Faculty of Medicine Novi Sad works on introducing *in situ* approaches in the analysis of respiratory mitochondrial function in skinned muscle fibers of human cardiac tissue.
Key words: Mitochondria; Energy Metabolism; Oxidative Phosphorylation; Cell Physiological Phenomena; Myocytes, Cardiac; Cell Respiration

Sažetak
Mitohondrije su sveprisutne organele eukariotskih ćelija i predstavljaju glavno mesto za stvaranje energije u formi adenozin trifosfata kroz proces oksidativne fosforilacije. Analiza i procena mitohondrijalne funkcije je od velikog značaja za izučavanje intraćelijskog energetskog metabolizma, mehanizama programirane ćelijske smrti, signalnih puteva, skladištenja kalcijuma i patofiziologije širokog spektra bolesti čoveka uključujući različite forme neurodegenerativnih oboljenja, miopatija, metaboličkog sindroma i kancera. Analiza respiratornog kapaciteta pokriva jednu od mnogih uloga koje mitohondrija igra u živim ćelijama i pruža nam važne podatke o funkcionalnom integritetu mitohondrija. Ocena pojedinih kompleksa respiratornog lanca ili drugih mitohondrijalnih enzima se uveliko primenjuje u proceni mitohondrijalne funkcije ili disfunkcije, međutim ona zanemaruje uticaj kompleksne funkcionalne i strukturne povezanosti mitohondrijalnih enzima i proteina i plazmatskih komponenti. Druga metoda ističe važnost izučavanja intaktnih mitohondrija u *in vitro* uslovima. Iako ova metoda ima brojne prednosti, u pojedinim aspektima funkcionalne ocene mitohondrija ona je nepotpuna. Sa aspekta proizvodnje i potrošnje energije, srčani mišić je izrazito zahtevno tkivo i upravo je očuvan rad mitohondrija preduslov za ostvarivanje njegove uloge. U saradnji sa Medicinskim fakultetom u Splitu i pod mentorstvom prof. dr Marka Ljubkovića, Katedra za fiziologiju na Medicinskom fakultetu Novi Sad radi na uvođenju metode *in situ* procene mitohondrijalne respiratorne funkcije na diseciranim mišićnim vlaknima humanog srčanog tkiva.
Ključne reči: mitohondrija; energetski metabolizam; oksidativna fosforilacija; fiziološki fenomen ćelija; srčana mišićna vlakna; ćelijska respiracija

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We would like to thank Professor Dr. Marko Ljubkovic from the University of Split School of Medicine for his unreserved sharing of knowledge and experience. It is indeed the most valuable thing that young researchers can get from their menhors.

The role of mitochondria

By their morphological appearance mitochondria are perhaps the most intriguing cell organelles. Practically, a mitochondrion is a balloon inside the balloon where inner and outer membranes create two

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Abbreviations
ATP – adenosine triphosphate
ADP – adenosine diphosphate
DNA – deoxyribonucleic acid
LPS – lipopolysaccharide

separate internal compartments: intermembrane space and matrix space [1]. Mitochondria are the major site of adenosine triphosphate (ATP) synthesis thus the life of a cell is dependant of their proper function. The inner membrane has several protein complexes attached to it or floating close to the membrane. Protein complexes create a respiratory chain which uses voltage and pH gradients to eventually generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. Most mitochondrial complexes and structural proteins are encoded by nuclear deoxyribonucleic acid (DNA) and translated in cytoplasmic ribosomes whereas mitochondrial DNA accounts for a relatively small fraction of total mitochondrial proteins. These complexes are known as nicotinamide adenine dinucleotide (NADH): ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II), ubiquinol-cytochrome c oxidoreductase (complex III), cytochrome c oxidase (complex IV) and ATP synthase (complex V). It is the travelling of electrons from one complex to another that generates redox energy in a single transfer. The released energy is used to pump the protons into the intermembranous space creating high concentration of protons in this compartment. Ultimately, complex V uses this proton-motive force that transfers hydrogen ion through its F0-F1 channel and produces ATP from ADP and inorganic phosphate [2–4].

Mitochondria also serve as an intracellular Ca^{2++} storage that can be possibly released under physiological conditions. At last, mitochondria play a central part in the process of programmed cell death where certain external or internal signals initiate pathways for the highly regulated suicide of the cell [1, 5].

The tissue that relies heavily on these ATP machineries that mitochondria are is the cardiac muscle. During a lifetime cardiomyocytes must contract repetitively at different frequencies. This means that they ought to have continuous supply of O_2 and high mitochondrial density which guarantee rapid oxidation of substrates and production of ATP to meet energy demands of the tissue [5].

The role of Mitochondrial Dysfunction

As a system is getting more complex, chances for errors to occur are increasing with every subunit being a potential site of mishap. Electron leakage in respiratory chain serves as the main source for generating reactive oxygen species and free radicals (superoxide anion radical $O_2^{\cdot-}$, hydrogen peroxide (H_2O_2) molecules produced by superoxide dismutase). This intrinsic pathway of increased production of reactive oxygen and nitrogen species may lead to structural abnormalities and functional breakdown in this organelle. In addition, other stimuli such as toxicants,

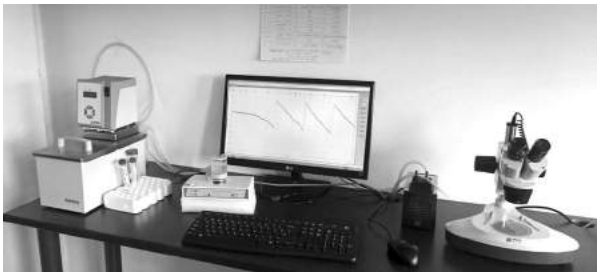

Figure 1. Laboratory for cellular Physiology
Slika 1. *Laboratorija za ćelijsku fiziologiju*

protein degradation products and stress on other cofunctioning organelles also affect mitochondrial function and may induce formation of mitochondrial permeability transition pores that is an initial step to apoptosis [6].

The number of publications in the field of mitochondrial function analysis is increasing rapidly and it serves as a proof that the role of mitochondria in the pathogenesis of various conditions is being placed in the center of interest to many scientists and research teams. The research of T. Boczek et al. shows that alterations in type 2 and 3 isoforms of Ca^{2+}-ATPase perturb energy-generating pathways and mitochondrial activity in a direction that is trying to maintain the levels of ATP production [7]. Macarini JR et al. investigated the effect of carnosine accumulation on mitochondrial function in isolated rat skeletal muscle. They found that acute carnosine exposure altered mitochondrial function and it is important for future studies of carnosinase deficiency, a disease that affects several human tissues [8]. The effects of exposure to bacterial endotoxine lipopolysaccharide (LPS) were studied in hepatic cell line HepG2, primary hepatocytes and on mitochondria isolated from the quadriceps muscle of pigs by Jeger V et al. They showed that LPS is capable of influencing mitochondrial function by either changing the membrane potential or altering the respiration [9]. Lastly, in their review paper, Su J et al. analyzed the interaction between endoplasmic reticulum stress and mitochondrial dysfunction in tumors during the development of chemotherapy resistance. This interaction was mediated by Bcl-2 family of proteins [10].

Understanding of mitochondrial malfunctioning in various pathological conditions, genomic background and molecular mechanisms may provide new therapeutic approaches for prevention of diseases associated with the defected function of mitochondria.

Analysis of Mitochondrial Respiratory Capacity

Analysis of mitochondrial function has become central in the basic research of mitochondrial physiology and the diagnosis of many diseases. Nearly half a century ago, Britton Chance and G.R. Williams isolated intact and active mitochondrial preparations and performed measurements of mitochondrial function [11, 12]. Nowadays the standard procedure of

isolating intact mitochondria is based on differential centrifugation of tissue or cell homogenates [13]. In spite of being helpful in assessing mitochondrial functional integrity, maximal capacity of oxidative phosphorilation and import of mitochondrial proteins, this method has several limitations:

– Mitochondria are affected by the isolation process. It is even more emphasised when the tissue specimen is affected by pathological process [14].

– Biased selection during differential centrifugation - swollen mitochondria have decreased density so the process of centrifugation will favour intact mitochondria. The proportion of diseased mitochondria may be significantly decreased compared with the initial sample [14].

– It demands larger quantities of cells (>200x10^6 cells) or tissue (above 500mg wet weight) [13].

– Natural interactions of mitochondria are lost in isolated mitochondria. As a consequence, functional properties of mitochondria are totally different *in vivo* and *in vitro.*

Studies of individual mitochondrial enzymes or respiratory chain complexes are widely used for assessing mitochondrial function. On one hand, breaking down mitochondrial architecture and estimating structure and function of complexes and subunits one by one provides useful information. On the other hand, those information are incomplete for they will not reveal how all those enzymes and complexes interact with each other in their physiological environment [2].

In vivo analysis of mitochondrial respiratory capacity, among other parameters of cellular energetics, is certainly the most physiologically relevant approach to mitochondrial studies. But unfortunately, the analytical scope for studying mitochondrial function is restricted to measuring mitochondrial redox state, membrane potential, calcium and reactive oxygen species [2].

In situ analysis combines some advantages of the previously mentioned methods while avoiding limitations of isolated mitochondria. It allows the assessment of mitochondrial function in an integrated cellular system, in a physiological environment: normal intracellular position and assembly, preserved interactions with cytoskeleton, nucleus and endoplasmic reticulum, intact multienzyme system of intracellular energy transfer. It resembles the situation in living cells more than the analysis of isolated mitochondria does [4].

The tissue sample is first mechanically dissected under the magnifier using micro tweezers to manipulate with the tissue and to eliminate unnecessary parts. Connective and fat tissue are removed. The second step is the application of special permeabilizing chemicals that specifically interact with cholesterol from membranes of cells or muscle fibers, attacking the cholesterol rich membranes such as plasmalema, while sparing the membrane of endoplasmic reticulum and mitochondrial membranes. Chemical permeabilizing agents like saponin, digitonin, filipin, α-solanin, α-tomatin or β-escin selectively attack the plasma membrane of muscle fibers, leaving the organelles and the cytoskeleton intact. After finishing the initial steps for tissue preparation, the mitochondrial respiratory capacity of the tissue sample is tested using the Oxygraph Plus system (Hansatech Instruments ltd Narborough Road, Pentney, King's Lynn, Norfolk, UK) **(Figure 1).** The Oxygraph Plus system consists of a highly sensitive S1 Clark Type polarographic oxygen electrode disc mounted within a DW1/AD electrode chamber and connected to the new Oxygraph Plus electrode control unit. It provides personal computer control of oxygen uptake or evolution measurements across a broad range of applications from studies of mitochondria and cellular respiration.

Figure 2. Steps in preparing the tissue sample for measurement
Slika 2. *Koraci u preparaciji tkiva za merenje*

At the Department of Physiology of the Faculty of Medicine in Novi Sad we are currently measuring normal mitochondrial respiration capacity in the rat heart and Guinea pig heart following the *in situ* method **(Figure 2).** After completion of studying animal models, as a part of our training process, we will turn our attention to human physiology, more specifically the human cardiac tissue. Our colleagues from Split utilized this method and published a couple of articles whose primary subject was the influence of various active substances on the respiratory function of mitochondria. The goal of their study was to assess the effect of trimetazidine on mitochondrial substrate oxidation directly in left ventricular myocardium with coronary artery disease patients [15].

Conclusion

The number of publications in the field of mitochondrial function analysis is increasing rapidly and it serves as a proof that the role of mitochondria in the pathogenesis of various conditions is being placed in the center of interest to many scientists and research teams. In the future our research will focus on investigating the role of mitochondria in the pathogenesis of human heart diseases and how various comorbidities have repercussion on the human heart tissue and mitochondrial respiratory capacity.

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COMPLICATIONS OF ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTIONS

KOMPLIKACIJE REKONSTRUKCIJE PREDNJE UKRŠTENE VEZE

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Summary

Introduction. The aim of study was to analyze the results and complications of anterior cruciate ligament reconstructions, treatment and prevention possibilities. **Material and Methods.** We performed 210 operations: 175 with patellar tendon graft and 35 with hamstring tendons. Men were 7 times more present in our survey. The average age being 27.1 years. The average follow up was 3.2 years. The participants answered a modified Knee Ostedoarthritis Outcome Scale questionnaire set. We used clinical examination tests (Range of motion, Lachman and pivot shift) and postoperative X-rays. **Results**. The complication's rate was 9% (19 patients). In patellar tendon group we registered one patellar fracture and pulmonary tromboembolism and two cases of cyclops lesions. Septic arthritis was more common among professional athletes and in hamstring tendons group. Reoperations had to be performed: in 5 cases of infections (continous irrigation and drainage), two arthrofibrosis and one osteosynthesis of patella. Graft had to be removed in 2.5% of cases and revisions happened mostly because of impropriate position of bone tunnels and new trauma. One algodystrophic syndrome happened in hamstring tendons group. Complications were successfully treated in 12 cases but 5 patients had knee contractures, so average Knee Ostedoarthritis Outcome Scale score was 88.2 points in comparison to 93.8 points in patients without complications. Seventy-three percent of patients returned to nonrestricted (sport) activities. Complications can compromise the final results of anterios cruciate ligament reconstructions. We registered similar complication rates between different grafts. Some of complications had almost every eleventh patient that resulted with reoperation at every eighteenth of them. **Conclusion**. The most of complications can be prevented and treated successfuly althought many questions are still left opened.

Key words: Anterior Cruciate Ligament Reconstruction; Intraoperative Complications; Postoperative Complications; Treatment Outcome; Lysholm Knee Score; Tendons; Bone-Patellar Tendon-Bone Grafting

Sažetak

Uvod. Cilj ove studije je analiza rezultata i komplikacija rekonstrukcije prednjeg ukrštenog ligamenta kao i mogućnosti lečenja i prevencije. **Materijal i metode**. Izvršeno je 210 operacija: 175 operacija ligamenta čašice i 35 operacija tetiva zadnje lože. Muškarci su bili sedam puta zastupljeniji u ovom istraživanju. Prosečna starost iznosila je 27,1 godinu. Prosečan period praćenja iznosio je 3,2 godine. Ispitanici su odgovarali na modifikovani *Knee Ostedoarthritis Outcome Scale questionnaire set* upitnik. Korišćeni su testovi za kliničko ispitivanje (obim pokreta zgloba, Lahmanov i pivot šift (shift) test) kao i rendgenski snimci. **Rezultati**. Stopa komplikacija iznosila je 0,9% (19 pacijenata). U grupi pacijenata kojima su operisani ligamenti čašice, registrovali smo prelom čašice, tromboembolijsku komplikaciju i dva slučaja ciklops lezije. Septični artritis je bio češći među profesionalnim sportistima i u grupi pacijenata kojima je izvršena operacija tetiva zadnje lože. Reoperacija je vršena: u pet slučajeva zbog infekcije (kontinuirana irigacija i drenaža), dve zbog artrofibroze i jedna zbog osteosinteze čašice. Kalem je morao biti uklonjen u 2,5% slučajeva a revizije su vršene uglavnom zbog neadekvatne pozicije koštanih tunela ili nove traume. Jedan algodistrofični sindrom dogodio se u grupi pacijenata kojima su operisane tetive zadnje lože. Komplikacije su uspešno izlečene u 12 slučajeva ali je pet pacijenata imalo zaostalu kontrakturu kolena, te je prosečan rezultat primenjenog upitnika iznosio 88,2 u poređenju sa 93,8 kod pacijenata bez komplikacija. Neograničenim sportskim aktivnostima vratilo se 73% pacijenata. Komplikacije mogu ugroziti konačni rezultat rekonstrukcije prednjeg ukrštenog ligamenta. Registrovali smo slične stope komplikacija među različitim kalemovima. Neke od komplikacija imao je skoro svaki jedanaesti pacijent što je rezultovalo reoperacijom kod svakog osamnaestog. **Zaključak**. Većinu komplikacija je moguće sprečiti ili uspešno lečiti, iako su mnoga pitanja još uvek ostala otvorena.

Ključne reči: rekonstrukcija prednjeg ukrštenog ligamenta; intraoperativne komplikacije; postoperativne komplikacije; ishod lečenja; Lisholmov skor; tetive; BTB graft

Introduction

Anterior cruciate ligament (ACL) injury was the main reason for ending a sport's career fourty years ago because it presents the beginning of the end of knee joint due to its instability [1, 2]. As arthroscopy and operative techniques developed ACL reconstructions provided excellent results without many complications of: bleeding, infection, scarf tissue, reduced operative time and allowed early rehabilitation [3]. The choice of graft for ACL substitution is also reduced into two most often used autografts: bone-pattelar tendon-bone (BTB) [4] and hamstring tendons [5]. Allografts are not abandoned

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Abbreviations

ACL – anterior cruciate ligament
BTB – bone-patellar tendon-bone
BHB – bone-hamstring tendons- bone
KOOS – Knee Osteoarthritis Outcome Scale
ROM – Range of motion

but rarely used nowadays [6]. Drilling the femoral tunnel through anteromedial portal is substituting former transtibial way, which can not provide the anatomic position (footprint) and results with instability [7, 8]. Authors satisfied with a single-bundle technique [5, 9, 10] still want to achieve developement, expecially in the field of graft fixation. One of those attempts represents a hybrid fixation of bone-hamstring tendons-bone graft with additional screw in tibia [9, 10]. A few decades ago it seemed logical that ACL reconstructions can prevent degenerative changes in knee joint, but later studies did not comfirm it [2], althought they can: restore knee stability, reduce later meniscal injuries, provide return to sports activities and improve the life quality of patients [9, 11, 12]. Dilemma still preasent is does double-bundle technique provide rotatory stability [13] and significantly better results than single-bundle technique [14]. Some authors achieved better rotatory stability with additional reconstruction of antero-lateral ligament [5]. Questionable are also ideal timing for operation [15] and the choice of rehabilitation protocol [16].

Reconstruction of ACL is very popular procedure, because the success rates between 80 and 90% of all operations [1, 4, 8, 17], with small percents of complications [1, 4, 5, 8, 17]. The number of patients with complications increases as it increases the number of operations proportionally. Various factors have been associated with failure after a primary ACL surgery and are divided into [4]:

– preoperative [15, 16]: improper diagnosis, poor indications, improper preoperative range of motion, improper surgical timing, failure to prepare for concomitant procedures, failure to note concurrent diagnoses;

– intraoperative [8, 18]: improper graft choice, graft harvest errors, inadequate notchplasty, improper tunnel placement, femoral tunnel blowout, dropped graft, graft laceration, graft-construct mismatch, screw-tunnel divergence, improper tensioning, inadequate graft fixation;

– postoperative [8, 19]: infection, loss of motion/stiffness, extensor mechanism failure, graft failure, patellar pain, deep venous thrombosis/pulmonary embolus.

The aim of this study was to: compare the complications of ACL reconstructions between different grafts, analyse treatment and prevention possibilities, because those complications can compromise the final operative results and decrease the life quality of patients.

Material and Methods

We used a retrospective multicentric study to follow postoperative results and complications on 210 patients. BTB graft was used in 175 patients while 35 of them had reconstruction with bone-hamstring tendons-bone (BHB) graft. The same surgeons operated both groups of patients.

All reconstructions were performed through anteromedial portal with attention to achieve isometric characteristics of ACL and anatomic position on footprint. Single bundle autografts were fixed by interference screws and tensioned with force of 80 N. There was no significant difference between groups considering age, but there was resuming the level of sports activities. We reconstructed ACL with hamstring tendons in women and some recreative sportsmen, while BTB graft was used in all professional athletes.

The second generation of cephalosporins (or clindamycin in cases of panicillin allergy) was used as antibiotic profilaxis, preoperatively and two days postoperatively [20]. If infection occured the most often used antibiotic was vancomycin, according to bacteria cultures after the aspiration of knee joint. We did not use thromboprophylaxis as a routine. The hospitalization time of patients without complications lasted 5–7 days, while in cases of infections it was three weeks on average. Modified Shelbourn's rehabilitation protocol [16] was used in all of cases.

The average age was 27.4 years (15–54). We operated 7 times more often men than women (183:27). The follow up was 2.0–6.5 years (3.2 on average).

Sports activities caused 87% of all ACL ruptures (182 patients), while 28 (13%) were injured mostly during traffic accidents and other every day activities. Concidering level of sports activities, 103 athletes (56.6%) were recreational ones, while active in sports clubs were 79 (43.4%) of them. Soccer is sport that caused twoo thirds of all injuries - 120 (66%). The second is basketball 11 (6%), then volleyball 9 (5%), martial arts and skiing both 8 (4.4%), handball 6 (3.3%), american football 3 (1.7%) and others in minor percentage.

Right knee was operated in 106 patients, left in 101, while three patients had nonsimultaneously bilateral ACL reconstructions. Four years pasted on average between the first and the second ACL reconstructions.

Anterior cruciate ligament was the only injured structure of knee joint in 108 cases (51%), 53 (25%) had rupture of medial meniscus; 48 (24%) of medial colateral ligament; 38 (19%) had serious cartilage damage; 15 (7.5%) lateral meniscus; both menisci were ruptured in 7 (3.5%) of cases; lateral colateral ligament in 12 (6%). We also performed one posterior cruciate ligament reconstruction.

The average period that lasted from injury to operation was 9.5 months (3 weeks to 17 years), because patients spent too much time on: first examination, diagnostics, waitings lists and postponed operation. More than 60% of them (mostly active athletes) were operated within the first four months after injury.

Our study included subjective symptoms and clinical examination: range of motion and func-

tional tests. The basic criteria for failure of operation were: postoperative instability (positive Lachman test over 6 mm in comparison to uninjured knee and positive pivot shift test) [21], or persistant swelling, pain and limited Range of motion (ROM) that unabled the patient to return to the level of activities he/she had before the injury.

We analyzed the position of bone tunnels and screws by X-rays with Staubli's and Rauschning's [22]. The best results were achieved if femoral tunnel was not vertical and placed as much posteriorly.

The results were showed by modified Knee Osteoarthritis Outcome Scale questionnaire set (KOOS) and Lysholm scores [23]. Patients filled as questionnaire sets. The KOOS questionnaire is divided into five parts: the first involves the quality of life following ACL surgery; the second one related to pain in different activities; the third is related to daily activities performed by the patient; the fourth is related to the level of physical activity, (Lysholm score), the fifth focuses on the very consciousness of the patient's quality of life and how he perceives injury.

This study excluded those patients who did not fill the questionnaire and undergone rehabilitation protocol.

All of complications were devided [24] into: intraoperative (connected to surgical technique and instruments) and postoperative. During the first three postoperative days we also followed the complications connected to anesthesia. Later on connected to improper surgery, rehabilitation, new trauma etc. Complications are analyzed, compared between groups and showed by **tables** and **figures**. We also showed the our possibilities of prevention and treatment.

Results

We registered some of the complications in 19 patients (9%). There was no big difference between different graft groups (8.6% BTB vs. 11.4% BHB group) (**Table 1).**

Early postoperative complications are shown by **Table 2**. Patients with general anesthesia had significantly less complications, although they had severe pain in operated knee. Patients with spinal anesthesia more often had blader disfunction and headache.

We registered excellent position of bone tunnels in 151 case (71.9%), good in 57 (27.1%), while only in 2 cases (1%) intolerant (too anterior) .As we tried to achieve as much posterior position of femoral tunnel, we also had a complication of perforation of posterior femoral cortex (**Figure 1**).

Intraoperative complications where the choice of graft did not have influence are also presented (**Figure 2).** They were connected to breakage of instruments (one case of drill's and guide's breakage) and one case of dropping the graft on the floor of operation room (with no infection).

The criteria for postoperative contracture of knee joint was limited ROM: flexion more than 20 degrees and extension more than 5 degrees. Five patients had stiffness. This complications occurs if they delayed operation 17 years after the injury, so proper ROM could not be achieved.

The most common complications were infection (2.9%) and graft's rupture (2.4%). Infection happened in 6 cases. Staphylococcus aureus was isolated three times, Staphylococcus epidermidis or lungdunensis, Klebsiela and Enterococcus once. One patient had obvious infection although bacteria was not found. All of the infections had symptoms within the first 10 days after the reconstruction, with: increased laboratory values of C-reactive protein (over 150), severe knee pain and increased body temperature. We treated them with urgent aspiration, irrigation, drainage and three weeks with antibiotics, untill symptoms disappeared and CRP decreased to physiological values. Infections were managed by continuous irrigation and suction drainage in four cases. Five out of six (83%) of them

Table 1. ACL complications depending of graft
Tabela 1. *Komplikacije u vezi sa kalemom*

Complications/*Komplikacije*	175 - BTB/ *kost-čašična veza-kost*	35 - BHB/ *kost-tetiva hamstringa-kost*	210 - Total/*Ukupno*
Algodystrophic syndrome/*Algodistrofični sindrom*	0	1	1 (0.5%)
DVT, PTE/*DVT, PTE*	1	0	1 (0.5%)
Arthrofibrosis/*Artrofibroza*	2	0	2 (1%)
Infection/*Infekcija*	4 (2.3%)	2 (5.7%)	6 (2.9%)
Graft failure/*Propadanje kalema*	2	0	2 (1%)
Loose body/*Opušteno telo*	1	0	1 (0.5%)
Rerupture/*Ponovno pucanje*	4 (2.3%)	1 (2.9%)	5 (2.4%)
Patellar fracture/*Prelom čašice*	1	0	1 (0.6%)
Total %/*Ukupno %*	15 (8.6%)	4 (11.4%)	19 (9%)

BTB - *kost-čašična veza-kost,* BHB - *kost-tetiva hamstringa-kost*, DVT - deep vein thrombosis/*tromboza dubokih vena, PTE - pulmonary thromboembolism/plućna tromboembolija*

Table 2. Complications connected to anesthesia
Tabela 2. *Komplikacije u vezi sa anestezijom*

Complication/*Komplikacija*	General/*Opšta*	Spinal/*Kičmena*
Blader disfunction/*Disfunkcija bešike*	0%	18 (9%)
Severe knee pain/*Jak bol u kolenu*	66 (31%)	22 (10%)
Nausea/*Mučnina*	20 (10%)	12 (6%)
Shivering/*Drhtavica*	52 (25%)	26 (12%)
Dizziness and headache/*Vrtoglavica i glavobolja*	10 (5%)	46 (22%)

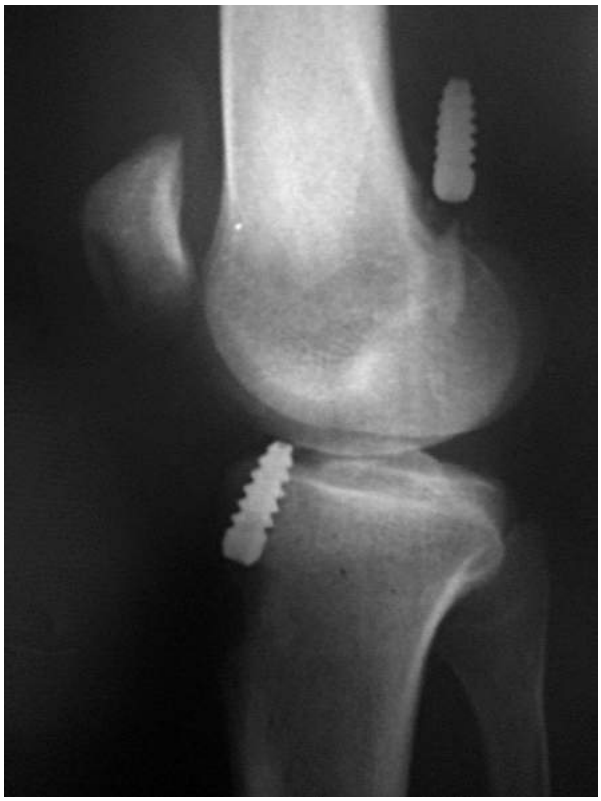

Figure 1. Perforation of posterior femoral cortex
Slika 1. *Prelom zadnjeg zida tunela u butnoj kosti*

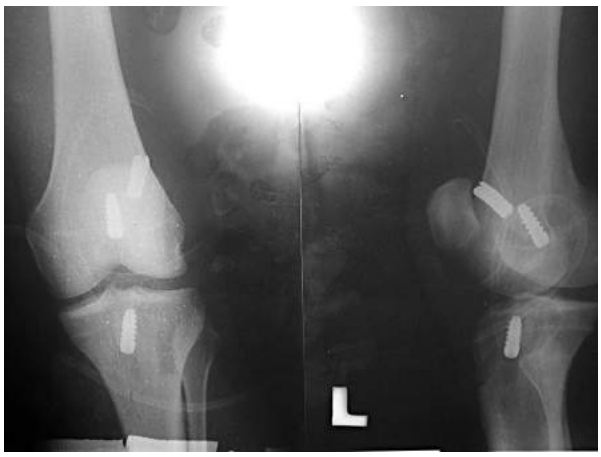

Figure 2. Broken drill in femur
Slika 2. *Slomljena burgija u butnoj kosti*

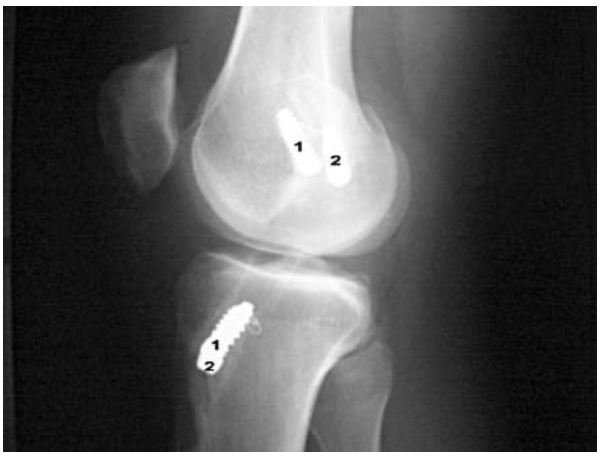

Figure 3. Revision implant-2, primar incorrect implant-1
Slika 3. *Ponovno postavljeni implantat – 2, Primarno nekorektno postavljen implantat – 1*

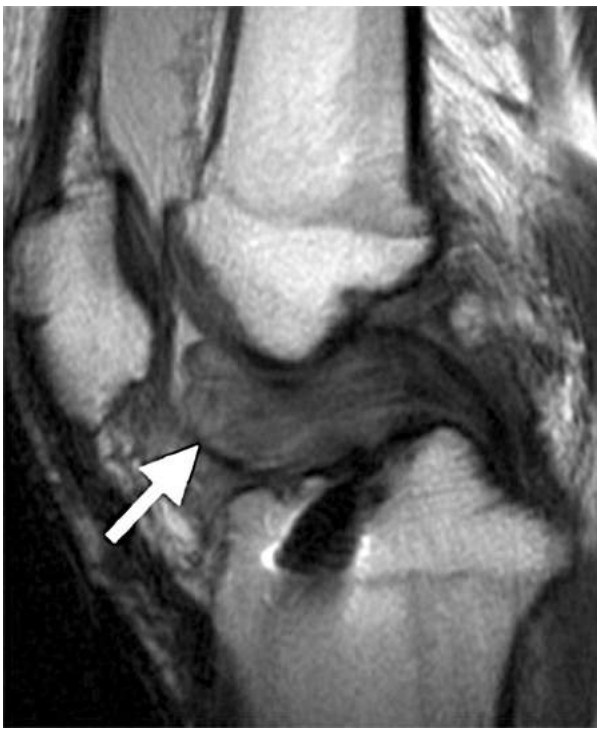

Figure 4. Cyclops (magenitc resonance imaging)
Slika 4. *Cyclops (snimak magentna rezonancija)*

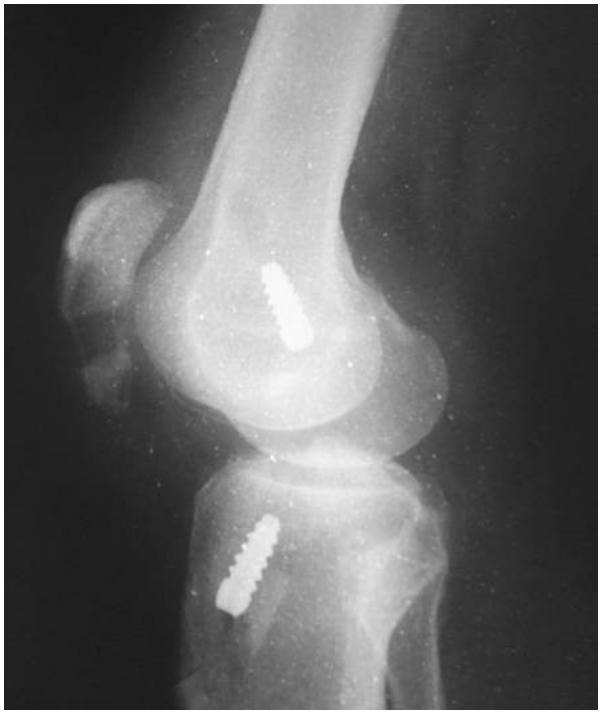

Figure 5. Patellar fracture after ACL reconstruction
Slika 5. *Prelom čašice posle rekonstrukcije prednje ukrštene veze*

were treated successfuly, but scores were lower (87 points on average).

We had to perform a revision surgery with graft substitution in 2.5% of cases. There were two cases of graft failure with unknown reasons among five patients. One of them did not want to be reoperated so he stopped with sports activities. The reasons for graft failure were: new trauma in two cases (although one patient participated a forbidden competition three months postoperatively) and too anterior femoral tunnel also in twoo cases (**Figure 3).** There was also a case of graft failure after treatment of infection. This resulted with instability so graft had to be removed and substituted with BTB graft from another knee. All of these patients had later return to sports activities (more than 9 months after revision).

Beside graft failure, the reasons for reoperations were: 6 mentioned infections, two arthroscopic treatment of cyclops lesions (**Figure 4**) and isolated cases of extraction of loose body (because of improper notch plasty) and open reduction and internal fixation of patellar fracture, one month after BTB reconstruction (**Figure 5)**.

Our patients had no complications such as: graft's impigemente, patellar's tendon rupture, maior vascular injuries and compartment syndrome. The worst outcome was in case of algodystrophic syndrome.

The functional scales showed significantly less points in patients with complications (average Lysholm score 89.0, KOOS 88.2) in comparison to patients without them (Lysholm 93.8 points, KOOS 95.1).

Seventy three percent of patients (153) has returned to non-restricted (sports) activities, while 52 (27%) has not. The objective reasons were: five cases of postoperative instability, pain and swelling in 11, limited flexion in twoo, limited extension in three and muscular weekness in twoo cases. Other 29 patients were not unsatisfied with operation result but they chainged their life style or they are afraid of new injury and operation. Return to every-day activities happened 6.5 months on average (3–12).

Discussion

The basic aims of ACL reconstructions are restoring of knee stability and returning to life activities on the level as it was before injury [1, 3, 5, 11, 12, 25]. This operative procedure is unsuccessful if knee joint is postoperatively unstable or stable but with limited ROM, pain or swelling [25]. The main reasons for reccurent instability are: surgical technique, problems connected with graft and other complications, while some of them remain unknown [8].

All of our reconstructions were performed with single-bundle technique. Yagi et al. [13] concluded that advantage of double-bundle is in 97% of restauration of anterior tibial translation, versus 89% in single-bundle; with rotator stability even higher 91%:66%. Despite this study, others [5, 26] did not find statistic difference between those techniques. Drilling twoo tunnels in both bones, difficult operation, expecially revisions, can compromise theoretic advantages of double-bundle technique. A group of French authors [5] tried to achieve rotatory stability in another way. They performed a combination of ACL reconstruction with antero-lateral ligament (ALL) at 83 patients and concluded that additional ALL reconstruction two years after operation provided discrete positive pivot shift test only in 7 patients while 76 of them had negative test. These authors claim that this operation provides better rotatory stability than double-bundle operation which also has more complications of cyclops lesia [26]. So, the first promising experimental double-bundle results [13] are not varified in praxis. According to Swedish National Register, that included 16.791 operated patients, there was no statistic difference between single and double-bundle techniques concidering KOOS scale and revision rates [14].

Mid third of patellar tendon with bone plugs (BTB) for a long period of time happened to be the golden standard for ACL reconstuction, but nowadays semitendinosus et gracilis tendons are more often in use [5, 26]. Hamstring tendons are popular as graft because of lower morbidity in the place of harvesting, decreasing anterior knee pain and extensor apparatus rupture [27]. Experimental advantage of BTB graft in strenght is not present if tendons are quadrupled [26, 27]. Hamstring graft has also some disadvantages. Potential complications such as: bone tunnel enlargement [27], problems with graft fixation [9, 10] and weekness of posterior thigh muscle group are registered. Since 2007 it was developed a method of hybrid fixation of ham-

string tendons with the attention to improve the results and reduce the complications of graft tensioning [9, 10]. That method was also used at some of our patients but we used more often BTB graft because of it's more uniformed results [17]. We indicate patellar tendon's graft in all sports active patients, while hamstring graft at women recreational patients and some professions with a lot of kneeling (automechanics, priests etc.).

The graft can be fixed with many invented devices, but we fixed it with domestic, titanium interference screws which caused no problems neither graft damage. Although they are the cheepest, there is no difference in knee stability between bioabsorbable and metallic screws [28]. Swelling and allergic reactions are less common after methal screws, while advantage of absorbable is easier postoperative MRI diagnostics [11, 36]. We did not register a signifficant difference between different graft's groups concidering complication rates (9:11%), neither in operative results (Lysholm scale BTB 93.9 points, BHB 93.1), which is similar to other results [1, 8, 17, 29].

Advantages of BTB graft are initial strenght and possibility of rigid fixation because of it's consistance of bone parts, why it is more often used in revision cases. We prevented potentional disadvantages of BTB graft in the following way:

– extensor mechanism's weakness was prevented with early mobilisation and passive extension during rehabilitation;

– patellar tendon's rupture did not happen in our study. Milankov et al. [30] had four cases among 2.215 BTB operations (0.18%). This complication can be prevented with adequate: harvesting of graft, sutures of tendon's sheath and avoiding local infiltrations of corticosteroids;

– patellar fracture had one of our patients (0.6%). It can be prevented with gentle harvesting with blade positioning 45 degrees over the patella. Other authors [31] had similar complication rate (0.45%) on much bigger specimen (10 cases out of 2.215 BTB operations).

– The advantages of STG graft are: less time needed for wound closure, small scarf, less painful place of harvesting tendons and possibility of use in double-bundle technique. We prevented potentional disadvantages of hamstring graft in the following way: - weak tibial fixation - with tendons harvesting with tibial periost and incorporation of cancellous bone [9];

– graft's strenght – with both tendons quadruppled and tensioning with the third screw (second in tibia-hybrid fixation);

– weakness of knee flexor muscles – with rehabilitation. Within two years after the operation patients do not have limited flexion also because of MRI varified regenerative possibility of hamstring tendons [32].

All of the factors that cause complications of ACL reconstructions are also devided into [33]: nonspecific (general) and specific. Nonspecific are: temporary anesthesiological, vascular and nerve injuries, infection, trombosis, algodystrophic syndrome etc.

Postoperative complications connected to anesthesia, similar to ours were registered by some other authors [34]. General anesthesia cause less bladder disfunction, dizziness and haedache, so we prefer it during the ACL reconstructions.

Blood vessels injuries are very rare. Insall et al. [33] published two cases of popliteal artery injuries, because improper operative techniques. Milankov et al. [31] had a rare case of medial inferior genicular artery's false aneurism.

Nerve injuries (n. saphenus i n. peroneus) are also rare and often temporary. They are usually caused if reconstruction lasts more than twoo hours under a turnique. We did not have this complication.

Haemathoma occurs because of injury of geniculate artery or vein or as a consequence of bleeding from bone tunnels. Prevention is adequate hemostasis and placing the wax on bone harvested places. Amount of wax layer should be thin because of potential granulomatosis reaction. The blood in knee joint (haemarthros) is common but temporary complication prevented by intraoperative drains and treated with postoperative punctions.

Extravasation of solution for arthroscopy can lead to compartment syndrome because leeking a fluid through holes in bursa and injured capsula. Knee joint should be flexed during the operation because flexed knee receive less fluid than extended.

Increased pressure of arthro-pump can also cause extravasation. Swelling most often dissapears within 4 weeks. If synovitis persists more than 6 months it is the complication connected with cartilage and menisci damage.

Infection is a rare complication in ACL reconstructions with autografts. The prevalence of septic arthritis after those operations has been reported to range from 0.15% to 6%, mostly under 2% [19, 35–40]. Infection can be acute (most often caused by Staphylococcus) or chronic (after 6 weeks). Some authors [35, 36], also had 6 cases of infections (2%), that happened more often in hamstring group. Staphylococcus was bacteria that caused around twoo thirds of all infections [19, 35–40]. We were lucky because our patients did not have Methicillin Resistent Staphylococcus aureus (MRSA). We successfuly treated infections with: aspiration, continual irigation, drainage and antibiotics according to antibiogram, but some grafts must be removed because of impregnation with purulent exudation [35–40]. The same authors [35–40] suggest that graft's extraction should be in following cases: if therapy is delayed, when graft is infected and cartilage is seriously damaged, when therapy efect is not satisfied, when bacterial cultures are persistently positive or infection can be spread if graft is not removed. Infection can be very serious complication expecially if we do not know a proper antibiotic, when bacterial culture is sterile although clinical signs are evident.

Risk population groups for septic arthritis after ACL reconstructions are: professional athletes, allergic to penicillin with immunosuppressive diseases

[19]. Some researches [39] showed that the prevalence of septic arthritis was 5.7% in the professional athlete population (88 of 1957 totally oparated). Our results also showed that active sportsmen are risk population for infection, by unknown reason. Preventive measures that should be performed are: aseptic preoperative treatment of operative field, aseptic conditions in operative rooms, irrigation of grafts before its placement in bone tunnels, experience of surgeon in early recognising and proper antibiotics. We registered more infections than it is expected (2.9%), similar to Yasen et al. [1], because we did not use all of mentioned measures. There are some evidences that 12% of microbiological examined grafts are initially contaminated, mostly with Staphylococcus [38], so we irrigated grafts before placement in bone tunnels. A group of Australian authors recently published no infections in 1300 ACL reconstructions with vancomycin pre-soaking of hamstring grafts [42]. There are also published incidents of dropping the graft during the operation [19, 38] and study [41] that surveyed leaders in sports medicine who perform ACL reconstructions to determine the preferred management when graft contamination occurs. Forty-nine of 196 (25%) surgeons reported at least one contamination during their career. Forty-three of the 57 (75%) contaminated grafts were managed with cleaning of the graft and proceeding with reconstruction, like we did. Ten (18%) were managed by harvesting a different graft, and 4 (7%) were substituted with an allograft. No infections in any of the contaminated grafts were reported because cultures do not correlate with clinical infections. If operative time lasts longer than 90 minutes those patients had three times more risk for infection [19].

Algodystrophic syndrome is also rare (0–0.4%) [1, 8] but serious complication because these patients have severe pain, vascular, nerve, trophic changes and limited ROM. We registered an isolated case in hamstring group, treated with redressement force, drugs and rehabilitation procedures.

Deep vein thrombosis (DVT) happen to be rare complication because the majority of patients are young healthy athletes (with average age of 27 years). We used thromboprophylaxis only in risk patients and registered one isolated case of pulmonar thromboemboly one month after the reconstruction with good outcome. A group of researchers from India [43] had twoo similar cases out of 112 patients, while Cullison et al. [44] one among 67. The most of authors do not recommend routine thromboprophylaxis [8, 17, 18, 43, 44], but there are some who claim that even five days of intrahospital thromboprophylaxis is not enough and that extended duration postdischarge therapy for 20 days only significantly reduces the incidence of DVT [45]. We do not agree because of increasing bleeding in knee joint and delayed rehabilitation.

Limited Range of Motion (LROM) and Patellofemoral Pain

The incidence of stiffness and pain after ACL reconstruction are very different in literature. While Shelbourne and Urch [46] indicated reintervention because of limited knee extension only at 1% of patients, Kartus et al. [47] on similar specimen of 604 patients had 13% of reinterventions. The majority of modern studies [1, 8, 17, 18, 46] did not register significant postoperative anterior knee pain and LROM. Some thirty years ago [47] there were published even 34% cases of anterior pain in BTB groups. In our study there was 5 cases of stiffness (2.5%). Some other authors [48] registered in 19% correlation between pain and flexion contracture. However, the majority of studies demonstrated no significant difference in range of motion following either BTB or ST reconstruction [20, 37, 38, 45, 46]. Limitation of extension used to be a problem after ACL reconstruction, particularly when using the patella tendon, but now, with early mobilization and passive full extension, the problem is minimized [48]. If it occurs despite propriate rehabilitation, arthroscopic surgery should be performed. The area of the scar should be identified and resected with a motorized shaver. The scar is usually anterior; if necessary, a notchplasty is done to regain extension. After the procedure, manipulation should release any extra-articular adhesions. Finally, if the motion is not complete after manipulation, the ACL graft should be removed. It is important to achieve the full range of motion desired while on the table since range of motion will not significantly improve with postoperative therapy [8, 29, 35].

Arthrofibrosis is defined as the presence of scar tissue in at least one compartment of the knee joint, leading to a decreased range of motion. Localized anterior arthrofibrosis, called "cyclops" lesion ranges from 4 to 35%, mostly under 10% [49, 50], as we had (only 1%). The worst complication that we registered was in BHB group, where a patient had LROM after arthrofibrosis with the following Sudeck's syndrome. Redressement force under anesthesia and rehabilitation did not provide an adequate result. Shelbourne et al. [49, 50] noticed that arthrofibrosis happens more often at patients operated within the first three weeks after the injury, as it was in our mentioned case. That was the reason why we waited for ACL reconstruction at least three weeks after the injury. If we rush into surgery too quickly, before patients get their motion back in the knee and swelling is reduced, a stiff knee may result. Some authors agree with that [49, 50], some do not, expecially nowadays, when there are published excellent outomes even if reconstruction is performed in first week after ACL injury [1, 29]. We can not have influence on the timing of operation if some patients come to be operated 17 years after the injury, with: global instability, osteoarthritis, muscle weakness and meniscus injuries. So, preoperative condition of knee joint have more influence on this complication than timing of operation [15]. Former dilemma concidering too much stretching of graft and possible rerupture because of aggressive rehabilitation are not proved [49]. Although, rehabilitation can not compensate a poor operation, which can leed to graft's impigemente in femoral notch [49]. This happens if it is positioned too

verticaly or anterior, after transtibial technique. The outcome is instability and rerupture. The prevention is femoral tunnel drilling through anteromedial portal, so we achieved anatomic position on footprint without such complication.

Reinterventions

Getelman et al. [6] concluded that the reason for 15% unsatisfied ACL reconstructions lies in: unrecognized colateral ligament injuries, development of rotatory instability, varus or valgus deformity or former meniscectomies. Early complications may occure within the first six months after the operation. They are mostly result of operation's techique, problems of graft's incorporation or too aggresive rehabilitation.

Late complications are caused by new trauma if a patients had full ROM and stabile knee. This happens at 5–10% of athletes [1, 25] and results with swelling and recurrent instability. Mistakes during the surgery are the most common cause of postoperative instability and non-anatomic position of graft in 70-80% of cases is the cause of bad outcome [8, 21, 50]. Improper tunnel position leeds to big changes of graft's tensioning during knee movements. The most common mistake is too anteriorly positioning a tunnel in femur. In that case, screw does not present too big problem during the revision, so it should not be extracted. There is enough space for a revisional screw (**Figure 3**). On the contrary, extraction of titanium screws can be a problem during the revisions when initial screw is good positioned. As we tried to achieve as much posterior position of femoral tunnel, sometimes without femoral guide (free hand), we had a complication of perforation of posterior cortex (**Figure 1**). Some other autors [51] think that in their 6 similar cases restricted rehabilitation protocol can be an alternative to revisional chirurgy. Although the returning to everyday activities is prolonged, they did not find the difference in functional recovery. Non-operative treatment was indicated in recreative athletes where time of returning is not crucial [51]. X-rays are good for the fast orientation for bune tunnels, but CT and MRI methods are more accurate, but more complicated [18, 52]. There is an agreement that improper position of graft can cause: complications, subjective symptoms, poor outcome and reintervention [8, 18, 51, 52]. We did not performed many revisions such Milankov et al. [8] did. They published 30 cases out of 2200 reconstructions (1.36%) and concluded that the reasons for ACL reoperations were: new trauma in 10 patients and improper tunnels in 15, while cause was unknown in 5 cases. Although they achieved very good results, the average Lysholm score was 85 points. Swedish National Register [14] contains 347 performed revisions in 16281 patients (2.1%) where ACL reconstruction was perfomed with single-bundle technique and 8 revisions among 510 patients (1.6%) in double-bundle group. We had 2.5% revisions, while a group of English authors [1] published 11.4% such of cases. These reoperations can be successfuly performed but with less excellent results than primar reconstructions [1, 8, 14].

If athletes return to sports activities without limitations, 2–10% of them have ACL injury of contralateral knee joint, most often within the first five years after initial reconstruction [11]. This may cause the end of a sport's carrer, because only 52% of them return to non-restricted sports activities after the both reconstructions [12]. ACL injury of contralateral knee is not a direct complication of the first operation, but it can happen because of sparing of the first operated knee.

Concidering returning to non-restricted activities, we achieved 73% of those cases. Results range between 40–88% in literature [5, 11, 12, 53]. According to average functional scales, we also achieved good average results among the patients without complications (Lysholm score – 94 points, KOOS – 95), comparable with others [1, 5, 8, 18], where Lysholm score ranges between 89 and 98 points, and KOOS from 88 to 95. The average values of the same scales are significanly lower in patients with complications (Lysholm – 89, KOOS – 88). The structure of complications (**Table 1**) is similar to some others [1], who had 9.2% of complications, with more common revisions (27 out of 237 operations – 11.4%).

The limitations of this study are connected to not using arthrometer for evaluation of tests in all of cases, potential subjectivity of patients during filling the qustionnaires and usage of X-ray method to analyse bone tunnels, which is not precise enough.

Study opens possibilities in the field of prevention complications and achieving better outcomes of ACL reconstructions. It also opens many questions such as: which is better type of graft, which is the ideal method of fixation and its tension.

Dilemma is when it is needed to sacrifice the graft for revision after the complication; is it enough to drain infected knee or to place continual irrigation. The aim of similar studies in the future could be to give answers why contaminated graft on the floor does not result with infection while a professional athlete without risk factors gets a septic arthritis. There is no enough evidence if tibial osteotomy can reduce re(rupture), in according to reduce posterior tibial slope [54] or does the reconstruction of anterolateral ligament provide greater rotatory stability. In the future we should find the answers of unreasonable instabilities, expecially if graft is well positioned. We still do not have answers why allergic patients to penicillin are in risk population for infection or why is every eight graft contaminated before its placing in bone tunnels. Unknown is also why we operate more often men in Balkan countries than women, when women have greater risk for ACL injury. There would be a revolution in sports traumatology if there would be invented reconstruction and rehabilitation protocol which would reduce the period to return to unlimited sports activities in less than 6 months without risk for complications.

Conclusion

Complications can compromise the final results of anterior cruciate ligament reconstructions, be-

cause functional scales are much lower in patients with complications than in those without them.

Every eleventh patient had some of the complications and every eighteenth had new operation because of them.

Similar complication rates were registered between different types of grafts, with significant difference only in more common infections in hamstring group.

General anesthesia provides less nonspecific early complications than spinal.

Prevention of limited range of motion can be solved with proper position of bone tunnels and rehabilitation. Intraoperative complications are depending of surgen's experience. Postoperative infections can be reduced by: intraoperative irrigation of graft, aseptic conditions and antibiotics. Thromboprophylaxis as a routine is not recommended.

The most of complications can be prevented and treated successfuly althought many open questions are still present.

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CURRENT TREATMENT OF ACUTE ISCHEMIC STROKE IN VOJVODINA

AKTUELNO LEČENJE AKUTNOG ISHEMIJSKOG UDARA U VOJVODINI

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Summary

Intravenous Thrombolysis in Acute Ischemic Stroke. Acute ischemic stroke is a major cause of mortality and morbidity in the world. Intravenous thrombolysis with recombinant tissue plasminogen activator remains the standard treatment for acute ischemic stroke for any patient presenting within 4.5 hours from symptom onset. However, it is more effective and safe when treatment starts early. This therapy for acute ischemic stroke has been administered in Vojvodina since 2008. Various factors influence the outcome after intravenous thrombolysis. Timely recanalization and reperfusion is associated with better clinical outcomes. **Mechanical Thrombectomy – a New Therapeutic Modality for the Treatment of Acute Ischemic Stroke.** Nevertheless, the rate of recanalization and favorable outcomes for patients with acute ischemic stroke due to large vessel occlusion are low after intravenous thrombolysis. In such patients mechanical thrombectomy has demonstrated significantly higher rates of recanalization and improved outcomes compared with intravenous thrombolysis alone. This endovascular reperfusion therapy began to be implemented in Vojvodina in 2016. **Conclusion.** Intravenous thrombolysis continues to play a key role in the treatment of all acute ischemic stroke patients, but mechanical thrombectomy should be the "gold standard" in the cases with large vessel occlusion.

Key words: Stroke; Brain Ischemia; Treatment Outcome; Endovascular Procedures; Thrombolytic Therapy; Thrombectomy; Tissue Plasminogen Activator; Reperfusion; Mechanical Thrombolysis

Sažetak

Intravenska tromboliza u akutnom ishemijskom moždanom udaru. Akutni ishemijski moždani udar je jedan od glavnih uzroka mortaliteta i morbiditeta u svetu. Intravenska tromboliza primenom rekombinovanog tkivnog aktivatora plazminogena je i dalje standard lečenja svih pacijenata sa akutnim ishemijskim moždanim udarom u prvih četiri i po sata od nastanka simptoma. Ipak, terapija je efikasnija i bezbednija, ako se lečenje započne ranije. Intravenska tromboliza u lečenju akutnog ishemijskog moždanog udara se u Vojvodini primenjuje od 2008. godine. Različiti faktori utiču na ishod nakon primene intravenske trombolize. **Mehanička trombektomija – nova terapijska opcija u lečenju akutnog ishemijskog moždanog udara.** Blagovremena rekanalizacija i reperfuzija su povezani sa dobrim kliničkim ishodom. Međutim, stepen rekanalizacije i povoljnog ishoda kod pacijenata sa akutnim ishemijskim moždanim udarom nastalim usled okluzije velikog krvnog suda, nizak je nakon intravenske trombolize. Kod ovakvih pacijenata primena mehaničke trombektomije je doprinela značajnom povećanju procenta rekanalizacije i poboljšanju ishoda u poređenju samo sa intravenskom trombolizom. Ova endovaskularna reperfuziona terapijska metoda je počela da se primenjuje u Vojvodini 2016. godine. **Zaključak.** Intravenska tromboliza i dalje ima glavnu ulogu u lečenju pacijenata sa akutnim ishemijskim moždanim udarom, ali mehanička trombektomija treba da postane „zlatni standard" kod pacijenata sa okluzijom velikih arterija.

Ključne reči: moždani udar; moždana ishemija; ishod lečenja; endovaskularne procedure; trombolitička terapija; trombektomija; rekombinovani tkivni aktivator plazminogena; reperfuzija; mehanička tromboliza

Introduction

Ischemic stroke (IS) is one of the leading causes of morbidity and mortality in the world. IS is also the leading cause of disability in the world, and, in addition to health-related consequences, it has enormous socioeconomic significance for the society as a whole [1]. Reducing the effects of stroke and improving outcomes is the goal of treating patients with acute IS.

Ischemic stroke is a result of occlusion of cerebral artery that leads to infarction of part of the brain, and clinically, to sudden development of focal neurological deficits [2]. In the acute phase of IS acute IS (AIS) around the central area of infarction, there is a zone of penumbra, not functional but still viable tissue, which can potentially be saved by a timely reperfusion. The precondition for reperfusion of the brain tissue in ischemia is recanalization of a previously

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Abbreviations

IS	– ischemic stroke
AIS	– acute ischemic stroke
IV	– intravenous
rtPA	– recombinant tissue plasminogen activator
IVT	– intravenous thrombolysis
NINDS	– National Institute of Neurological Disorders and Stroke
NIHSS	– National Institutes of Health Stroke Scale
SU	– Stroke Unit
CCV	– Clinical Cventer of Vojvodina
mRS	– modified Rankin score
MRI	– magnetic resonace imaging
sICH	– symptomatic intracerebral haemorrhage
NNT	– number needed to treat
OTT	– onset to treatment time
SITS	– Safe Implementation of Thrombolysis in Stroke
ICA	– internal carotid artery
AF	– atrial fibrillation
MCA	– middle cerebral artery
MT	– mechanical thrombectomy
MERCI	– Merci Retrieval System
ESO	– European Stroke Organization

occluded artery [3]. In this way, the size of the potential damage to the cerebral parenchyma can be reduced, neurological improvement can be achieved, and patient outcome after AIS can be improved.

Intravenous Thrombolysis in Acute Ischemic Stroke

Recanalization of a previously occluded artery can be achieved by intravenous (IV) administration of recombinant tissue plasminogen activator (rtPA), which remains the basis for treating patients with AIS [4]. Clinical efficacy of intravenous thrombolysis (IVT) was proved in 1995 after publication of the National Institute of Neurological Disorders and Stroke (NINDS) study [5]. This large randomized controlled study compared the AIS outcome in patients who received rtPA or placebo within 3 hours of the onset of symptoms. In the treated group, a relative increase in the percentage of patients with a favorable outcome was achieved, which meant the absence of disability after three months by 50% (modified Rankin score – mRS ≤ 1 after three months, 39% of the patients in the treated group compared with 26% of the patients in the placebo group). The following year, the Food and Drug Administration (FDA) approved rtPA in the treatment of AIS [6]. In 2008, the results of the randomized controlled study European Cooperative Acute Stroke Study III (ECASS 3) showed that the therapeutic effect and clinical benefit of IVT exist even if rtPA is administered over a period of 180–270 minutes from the onset of symptoms [7]. This study showed that in the time window of 3–4.5 hours after the onset of IS symptoms, IVT administration resulted in an absolute increase of the percentage of patients with a favorable outcome after three months of 7% (52% of treated patients had mRS ≤ 1 three months after AIS versus 45% in the placebo group). The symptomatic intracerebral haemorrhage (sICH) occurred in these two studies in 6% and 8% of cases versus 0.6% in the placebo group, but the therapeutic benefit significantly exceeded the risks [5, 7]. To date, several large multicentre, controlled, double-blind, randomized studies have confirmed IVT's efficacy in AIS, summed up in a single meta-analysis [8], which showed that the benefits of IVT use exist for all types of IS, regardless of the patient age, stroke severity, severity of neurological deficits expressed by the National Institutes of Health Stroke Scale (NIHSS), or the time of application of the therapy within the 4.5 hours time window.

Intravenous Thrombolysis for Acute Ischemic Stroke in Vojvodina

Intravenous thrombolysis with rtPA today represents a standard treatment of all patients with AIS within the first 4.5 hours after the onset of symptoms [9]. The first intravenous thrombolytic therapy in Serbia was performed in 2006 [10], and in Novi Sad two years later [11], when the first patient received IVT at the Neurology Clinic of the Clinical Center of Vojvodina (CCV). Since then, the number of patients treated with IVT has been increasing each year, to which contributed also the establishment of the new Emergency Center in the CCV, in which the Stroke Unit (SU) was formed. However, the percentage of patients who are treated with IVT today is only 5–8% of all patients who are examined for AIS in the CCV. The reason for this is, primarily, the small time window for the application of therapy [9], due to which the late recognition of patient's symptoms, delay in calling emergency services, i.e. arrival to the hospital after the time window of 4.5 hours, prevent the use of this therapy. These percentages are similar in most countries, and higher percentages (> 10%) of patients treated with IVT are recorded only in the most economically developed countries. Studies have shown that even if all patients with AIS would arrive within the appropriate time window, only one third would be eligible for IVT [9]. This may be explained by numerous contraindications, fear of complications, absence of the expected efficacy (severe clinical picture, high NIHSS score, occlusion of a large vessel, etc), but also by insufficient knowledge of both the general population and medical workers. A number of strategies have been considered to increase the number of patients with AIS who would be treated with IVT [9]. Today, it is known that in some conditions that were previously considered contraindications, such as seizures, age over 80 years, withdrawing symptoms and too mild or too severe stroke, IVT can be safely administered [8]. Recently, the American Heart Association and the American Stroke Association made clear recommendations on the use of IVT in the case of specific clinical conditions that can sometimes be considered relative contraindications [9]. In the case of a dilemma, the risk-benefit assessment should always be the first consideration in each individual case. Unknown time of symptom onset and the so-called wake-up stroke are still one of the main reasons for the decision not to perform IVT [12]. Magnetic

resonance imaging and diffusion-FLAIR mismatch techniques can enable selection of patients in whom the use of IVT would be justified.

With the help of telemedicine, doctors in outpatient centers who do not have access to the SU can be provided with expert assistance by neurologists and neuroradiologists when deciding on the application of IVT, which in many centers contributed to an increase in the percentage of patients with AIS treated with IVT, without increasing the risk of hemorrhagic complications [13]. Thanks to the Telestroke project, in some hospitals in Vojvodina the application of IVT under the supervision of the neurologists from the CCV started [14], and it is today routinely applied. In economically developed countries, mobile SU have been formed, thanks to which the therapy can also be applied in the field, but the justification of such models is still being tested [15].

Time of Initiation of the Therapy

Although IVT may be started within the first 4.5 hours following the onset of symptoms of IS, results of several studies have shown that the effect of therapy is time dependent and that the therapeutic response is better if the therapy is applied earlier [16, 17]. It has been shown that every additional 15 minutes of delay in IVT application reduces the chance of a patient's discharge or his/her independent walking at discharge by about 4% [16]. Also, with every 15 minutes of delay in IVT, the risk of sICH is increased for about 4% and for about 4% of fatal outcome [16]. Furthermore, it has been shown that it is needed to treat 4.5 patients with AIS by intravenous thrombolysis in order to have one patient fully recovered (number needed to treat - NNT) if the drug is applied within the first 90 minutes after symptom onset. The number of patients needed to be treated is twice as high (NNT-9) if the IVT is applied within a period of 90 to 180 minutes, and if applied after 180 minutes, NNT is 14.1 [18].

In order to give the therapy to patients with AIS as soon as possible, rapid recognition of the symptoms, prompt response of emergency medical service, and efficient treatment after the patient has arrived to the hospital are necessary [19]. The average time from the onset of AIS symptoms to the application of IVT, onset to treatment time (OTT), in industrialized countries is about 140 minutes [20], and it is recommended that the door to needle time (DNT) should not be longer than 60 minutes [18]. In our conditions, in the CCV these periods are 10–15 minutes longer than the above mentioned, which suggests that it is necessary to optimize and improve each stage in the management of patients with AIS.

Outcome after Administration of Intravenous Thrombolysis

Since patients in daily clinical practice differ from those included in large clinical studies, in 2002 the Safe Implementation of Thrombolysis in Stroke (SITS) registry was established in order to evaluate the outcome and complications in patients with AIS treated with IVT in daily clinical work. The results showed that three months after AIS and IVT application, 40-45% of patients were without any disability (mRS $\leq$ 1), and 55–60% were functionally independent (mRS $\leq$ 2) [20]. With the same goal, from the beginning of IVT administration in Serbia, the Serbian Experience with Thrombolysis in Ischemic Stroke (SETIS) registry was established [21]. In the CCV, the results showed that 43.5% of patients three months after AIS and IVT had mRS $\leq$ 1, while 55.1% of patients had mRS $\leq$ 2 [22], which is in line with the results of the SITS registry.

In most studies, the predictors of unfavourable outcome (mRS 3–6) at three months despite the administration of IVT were older age, a more severe clinical picture (i.e. a higher NIHSS score on admission), glycemic status on admission rate, OTT, presence of early signs of brain ischemia, proximal occlusion, and absence of early recanalization [23]. In patients in the CCV, the occurrence of recanalization was associated with early neurological improvement and a favourable outcome at three months [24]. Among the patients who had computed tomography angiographically verified recanalization of previously occluded cerebral arteries, 54.6% had an early neurological improvement; 72.3% were functionally independent (mRS $\leq$ 2) at three months, compared with 15.0% of patients with early neurological improvement and 30% with mRS $\leq$ 2 at three months in the group of those who did not have recanalization. In the analysis of specific clinical conditions, earlier reports showed that patients with occlusion of extracranial parts of the internal carotid artery (ICA) had a worse outcome than patients without an ICA occlusion [21]. Furthermore, patients with atrial fibrillation (AF) had a worse outcome than those without AF [25]. However, in both cases after regression analyses, it was shown that independent predictors of poor outcome were older age and clinical picture, i.e. the NIHSS score on admission. Also, by comparing the types of infarction according to the Oxfordshire Community Stroke Project (OCSP) classification, it was shown that the patients with larger threatening strokes had worse outcomes despite the use of IVT [26]. Today, it is known that a higher NIHSS score on admission indicates larger infarction, that is, more proximal occlusion, when the chance of recanalisation after application of IVT is smaller, and therefore a chance of neurological improvement and a favourable outcome at three months is lower [24, 26]. Several studies have shown that IVT has a modest effect in case of large vessel occlusion (terminal ICA, M1 segment of the middle cerebral artery (MCA) or basilar artery). The failure of IVT in proximal occlusions has also been explained by the size of the thrombus itself [27–30].

Although several studies have shown that IVT contributes to a better outcome in all IS subtypes and in the entire range of severity of clinical picture, it is now clear that IVT alone is insufficiently effective in proximal occlusion caused by a large thrombus [29]. A higher percentage of recanalization was achieved by the intraarterial endovascular approach and mechanical extraction of the thrombus after IVT, which is today the standard treatment of patients with AIS caused by large vessel occlusion [30].

Mechanical Thrombectomy - a New Therapeutic Modality for the Treatment of Acute Ischemic Stroke

The introduction of mechanical thrombectomy (MT) in the clinical practice marks the beginning of a new era in the treatment of AIS caused by the occlusion of large vessels in the frontal cerebral circulation.

The Food and Drug Administration approved the use of the first endovascular device: Merci Retrieval System (MERCI®) in August 2004 [31]. Using this device, complete recanalization was achieved in 48% of patients with AIS and large vessel occlusion, treated within the first 8 hours after symptom onset and in 60.8% of patients in combination with adjuvant intra-arterial thrombolytic therapy. The occurrence of sICH was recorded in 7.8%. Using the modern generation of MERCI devices, 69.4% of patients had recanalization with additional thrombolysis (intra-arterial or intravenous), with favourable clinical outcome in 34% of patients; however, there was no control treatment group in this study [32].

The initial optimism for MT was shaken when three large randomized controlled studies failed to determine the beneficial effect of endovascular treatment compared with IVT [33–35]. However, the designs of these studies were criticized: inadequate patient selection (in one of the studies), no necessary evidence of large vessel occlusion, use of older technology (mainly the first generation "retriever" devices) and a longer period of time until the start of endovascular intervention. Nevertheless, a post hoc analysis of subgroups with CT angiographically verified occlusion in the frontal circulation, showed statistically significant benefit of this endovascular treatment within 90 minutes after IV rtPA administration [36].

To date, nine positive large randomized controlled studies have been published that compared the results of treatment of AIS patients using MT after IVT or only with MT versus treatment with IVT alone [30, 37, 38]. Publishing the results of these studies, which used mostly the new generations of stent retriever, made major changes: the clear superiority of this type of endovascular treatment was demonstrated compared with standard IV treatment, both in terms of improving the percentage of recanalization and in terms of increasing the percentage of patients without disability at 90 days (mRS ≤ 2). Clear evidence of safety and efficacy of MT was also confirmed by the collaborative meta-analysis HERMES (Highly Effective Reperfusion Evaluated in Multiple Endovascular Stroke trials), which included the first five positive studies [39].

This meta-analysis showed that percentage of patients with good functional outcome (mRS ≤ 2) at 90 days was significantly higher in the group of patients treated with MT with or without prior IVT (46% of patients) than in the group of patients treated only with IVT (26.5%) [39]. In addition, the use of MT significantly reduced disability, i.e. the average mRS score at 90 days was reduced (adjusted cOR 2.49, 95% CI 1.76-3.53; p <0.0001). This meta analysis also showed that MT required the treatment of 2.6 patients in order to decrease the mRS by 1 or more points. Mortality in the first 90 days and the risk of sICH did not differ between patients who were treated with MT and IVT and those treated with only IVT. Another meta-analysis of the same five studies indicated that the clinical benefit of MT was also present if the intervention started within the first 7.3h (onset-to-expected arterial puncture time of 7 hours and 18 minutes) [40].

Current Recommendations for the Treatment with Mechanical Thrombectomy

Following re-evaluation of the AIS treatment protocol and the consensus opinion reached by members of the European Stroke Organization (ESO-Karolinska Stroke Update), supported by other European neurological associations at the end of 2015 [30], the following recommendations for the application of MT were given:

– Mechanical thrombectomy, in addition to IVT in the first 4.5 h, is indicated in case of occlusion of a large artery in frontal cerebral circulation up to 6h from the onset of symptoms (grade A, level of evidence 1a);

– Mechanical thrombectomy should not prevent IVT and vice versa, IVT should not delay the application of MT (recommendation grade A, level of evidence 1a);

– Mechanical thrombectomy should be performed as soon as possible and after establishing indications (grade A, level of evidence 1a);;

– If IVT is contraindicated (e.g. in Warfarin-induced therapeutic INR, >1.7), MT is recommended as the first therapeutic modality (recommendation level A, level of evidence 1a);

– Patients with acute occlusion of the basilar artery should be evaluated in centers with multimodal imaging, applying MT and IVT when there is an indication (grade C, level of evidence 4);

– The decision to initiate MT should be made jointly by a multidisciplinary team (neurologist, interventional radiologist, anesthesiologist) (grade C, level of evidence 5);

– The choice of anesthesia depends on individual situations; an effort should be made to avoid delaying MT (grade C, level of evidence 2b).

Patients with indications for MT are those in whom CT angiography has demonstrated a large blood vessel occlusion, primarily in the anterior circulation (M1 segment of the MCA or distal part of the ICA), NIHSS score on admission ≥ 6; Alberta Stroke Program early CT (ASPECT) score ≥ 6 [30].

Mechanical Thrombectomy for Acute Ischemic Stroke at the Clinical Center of Vojvodina

In the CCV, the era of the new therapeutic approach to AIS began in December 2016. So far, in the one-year period, a total of 17 AIS patients were treated with MT combined with IVT or without IVT, respecting the ESO-Karolinska recommendations [30]. The average age of our patients was 62 years. More patients were female (58% versus 42%). The average NIHSS score on admission was 14. The prevalence of risk factors

was as follows: arterial hypertension (92%), smoking (50%), atrial fibrillation (42%), hypercholesterolemia (42%), and diabetes mellitus (25%). The onset to puncture time was 246 minutes, which does not differ significantly from the results of other clinical studies [39, 40]. The percentage of successful recanalization was 82.4%. Assessment of clinical outcome in the first 90 days is one of the key indicators of successfulness of MT. A favourable clinical outcome depends on several factors. In the one-year follow-up period, 56.3% of patients had a favourable outcome (mRS $\leq$ 2).

Like after IVT, in the treatment of AIS by MT time remains the most important factor and key to success. Recent results suggest that any delay in achieving a 30-minute recanalization increases the risk of a worse clinical outcome by 12%, and that one-hour delay in achieving reperfusion reduces the chances of a favourable clinical outcome by 38%. The development of complications may also affect the outcome. The most severe periprocedural complications of MT are recurrent IS, occurring in about 6% of patients, and sICH, seen in about 8% [39, 40].

Conclusion and Future Guidelines

Intravenous thrombolysis continues to play a key role in the treatment of all patients with acute ischemic stroke. Today, there are quite obvious indicators that mechanical thrombectomy represents the "gold standard" in the treatment of patients with clinically most severe acute ischemic strokes due to occlusion of large blood vessels. The key challenge is to use this endovascular method in the safe and effective way. In the era of rapid development of endovascular devices for the treatment of acute ischemic stroke, it is necessary to make a balance between stricter patient selection and the benefits of treatment. Despite the varieties in time intervals, speed of revascularization and complications, our initial results in treating patients with mechanical thrombectomy are encouraging and do not differ significantly from the results of large studies.

However, there are fields that we can and must influence. In order to increase the number of patients with acute ischemic stroke who would be treated with this therapy and in order to apply the therapy as soon as possible, increased involvement is needed at all levels of management of acute ischemic stroke patients, as well as continuous education of the population. Provision of adequate re-education and cooperation with emergency medical teams is one of the prerequisites for the adequate treatment of these patients.

A significant clinical benefit can be achieved by relatively easy and simple implementation of what we already know: shortening the time from admission to the hospital to the application of recombinant tissue plasminogen activators. An improved coordination of the multidisciplinary team (neurologist, radiologist, anesthesiologist) would create conditions for shortening all significant time intervals until the initiation of endovascular intervention, i.e., until achieving the recanalization and reperfusion. With better selection of patients and greater experience, the shortening of the time from the onset of acute ischemic stroke symptoms to the application of therapy still remains the most important part of our efforts.

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GLUCOCORTICOID THERAPY AND ADRENAL SUPPRESSION

TERAPIJA GLUKOKORTIKOIDIMA I ADRENALNA SUPRESIJA

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Summary

Introduction. Adrenal insufficiency results from the inadequate adrenocortical conjunction. Adrenal insufficiency can be primary, secondary and tertiary one. The most common cause of adrenal suppression is the effect of exogenous therapy with glucocorticoids. **Glucocorticoids.** Corticosteroids are used in treatment of endocrine and non-endocrine diseases. They are applied as a substitution therapy in the patients with primary and secondary adrenal insufficiency. Due to their immunosuppressive and anti-inflammatory characteristics, they are used to treat a wide range of diseases. They are usually divided according to the length and size of the effect i.e. how they are applied. **Adrenal Insufficiency.** Glucocorticoid therapy may lead to a number of adverse effects such as a disorder in glucose metabolism, osteoporosis or frequent infections. Adrenal suppression is the most common complication resulting from corticosteroid application. The function of the hypothalamus-pituitary-adrenal axis may be inhibited for months after the treatment cessation. There are several predictors of potential glucocorticoid-induced adrenal suppression. **Diagnosing Adrenal Insufficiency.** The most frequent symptoms and signs of adrenal insufficiency are fatigue, nausea and vomiting, hyponatremia, hyperpigmentation or hypotension. Algorithm for the diagnosis of adrenal insufficiency must be followed in clinical practice. **Reduction in Glucocorticoid Therapy.** Reduction or complete cessation of the therapy is indicated when the maximum therapeutic benefit has been achieved or when considerable side effects, such as diabetes mellitus, severe hypertension, osteoporosis i.e. adrenal insufficiency, develop. **Conclusion.** Numerous synthetic glucocorticoids have been developed to be used in everyday clinical practice and they can be administered systemically or locally. A lot of side effects are associated with chronic administration of glucocorticoids. In order to avoid complications, it is recommended to administer intermediate-acting glucocorticoids every second day. In addition, the patients must be monitored carefully and glucocorticoid therapy should be discontinued gradually to prevent adrenal insufficiency or reactivation of the disease under therapy.

Key words: Glucocorticoids; Adrenal Insufficiency; Drug-Related Side Effects and Adverse Reactions; Diagnosis; Signs and Symptoms; Hydrocortisone

Sažetak

Uvod. Adrenalna insuficijencija posledica je neadekvatne adrenokortikalne sprege. Razlikujemo primarnu, sekundarnu i tercijarnu adrenalnu insuficijenciju. Efekat egzogene terapije glukokortikoidima je najčešći uzrok adrenalne supresije. **Glukokortikoidi.** Kortikosteroidi se koriste u lečenju endokrinih i neendokrinih oboljenja. Primenjuju se kao supstituciona terapija kod bolesnika sa primarnom ili sekundarnom adrenalnom insuficijencijom. Zbog imunosupresivnih i antiinflamatornih osobina koriste se u širokom spektru oboljenja. Najčešće ih delimo prema dužini i jačini dejstva, odnosno prema načinu primene. **Adrenalna insuficijencija.** Terapija glukokortikoidima može da dovede do brojnih neželjenih efekata – poremećaja metabolizma glukoze, osteoporoze ili čestih infekcija. Najčešća komplikacija prilikom upotrebe kortikosteroida je adrenalna supresija. Inhibicija funkcije hipotalamusno-hipofizno-nadbubrežne osovine, izazvana upotrebom kortikosteroida, može trajati mesecima nakon što je tretman obustavljen. Postoji nekoliko prediktora potencijalne glukokortikoidima indukovane adrenalne supresije. **Dijagnostika adrenalne insuficijencije.** Nadbubrežna insuficijencija manifestuje se skupom simptoma i znakova, a najčešće su to malaksalost, mučnina i povraćanje, hiponatremija, hiperpigmentacija ili hipotenzija. U kliničkoj praksi neophodno je pratiti algoritam dijagnostike nadbubrežne insuficijencije. **Smanjenje glukokortikoidne terapije.** Smanjene ili potpuno ukidanje terapije je indikovano kada je postignuta maksimalna terapeutska korist, ili kada se pojave značajni sporedni efekti poput dijabetesa melitus, teške hipertenzije, osteoporoze, odnosno nadbubrežne insuficijencije. **Zaključak.** Razvijeni su brojni sintetski glukokortikoidi koji se koriste u svakodnevnoj kliničkoj praksi koji se mogu ordinirati sistemski ili lokalno. Mnogi sporedni efekti su povezani sa hroničnom administracijom glukokortikoida. Da bi se izbegle komplikacije, preporučuje se terapija na drugi dan primenom glukokortikoida srednje dugog dejstva. Takođe, potrebno je pažljivo praćenje pacijenata i postepeno ukidanje glukokortiokoidne terapije kako bi se izbegla nadbubrežna insuficijencija ili reaktivacija bolesti pod terapijom.

Ključne reči: glikokortikoidi; adrenalna insuficijencija; nuspojave i neželjeni efekti lekova; dijagnoza; znaci i simpotomi; kortizol

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Abbreviations

HPA	– hypothalamic-pituitary-adrenal axis
ACTH	– adrenocorticotropic hormone
CRH	– corticotropin-releasing hormone
AI	– adrenal insufficiency
GCs	– glucocorticoids
11β-HSDs	– 11β-hydroxysteroid dehydrogenase
CS	– corticosteroids
COX	– cycloozygenase
NOS	– nitric oxide synthase

Introduction

Adrenal insufficiency results from the inadequate adrenocortical feedback i.e. the hypothalamic–pituitary–adrenal axis (HPA). It may be a result of insufficient function of the adrenal cortex – primary adrenal insufficiency (Addison's disease) as well as of insufficient secretion of adrenocorticotropic hormone – secondary adrenal insufficiency (the most frequent consequence of a pituitary gland tumor) or insufficient secretion of corticotropin-releasing hormone (CPH) by the hypothalamus – tertiary adrenal insufficiency (AI). Primary adrenal insufficiency may occur within polyglandular autoimmune syndromes. Since autoimmune adrenalitis is the most common cause of primary adrenal insufficiency, the presence of other autoimmune disorders which may require glucocorticoid therapy must be taken into consideration [1, 2]. In addition, drugs can induce adrenal insufficiency in several ways, such as by modifying their metabolism in the liver, binding to glucocorticoid receptors, by suppression of gene expression, by inhibition of steroidogenesis, due to the bleeding in the adrenal glands, by causing autoimmune hypophysitis or by suppression of HPA. Suppression of HPA axis is a well-known and tested effect of exogenous therapy with glucocorticoids (GCs) and GC therapy is the most common cause of adrenal suppression.

Synthetic Glucocorticoids

Glucocorticoid therapy does not necessarily lead to a disorder in HPA axis secretion, but it may cause the complete adrenal gland atrophy [3]. Since the introduction of GCs into the treatment of rheumatoid arthritis in 1949, the range of their therapeutic application has been widen considerably, including a great number of non-endocrine and endocrine diseases [4–6]. The clinical efficiency of synthetic glucocortioids depends on their pharmacokinetics and pharmacodynamics. It is known that the presence of 11 β -hydroxyl group is of great importance for anti-inflammatory and immunosuppressive effect. The presence of 11β–hydroxysteroid dehydrogenase (11β–HSDs) regulates the target cell adjustment between the active hydroxy- and inactive oxoform of a steroid [7]. Another very important pharmacokinetic characteristic of corticosteroids is that they bind to the plasma proteins, and only the unbound fraction is biologically active. As it is known, the endogenous cortisol is bound to the globulins up to 67% to 87% in humans, whereas it is bound to albumin from 7% to 19%. Therefore, about 95% of cortisol is bound to the plasma proteins. Except for prednisolone, synthetic corticosteroids bind predominantly to albumin and only marginally to globulin.

Corticosteroids (CS) are used to treat endocrine and non-endocrine diseases. First of all, they are applied as a substitution therapy in the patients with primary and secondary adrenal insufficiency as well as in the treatment of congenital adrenal hyperplasia [8, 9]. Due to their immunosuppressive and anti-inflammatory characteristics, they are used to treat a wide range of diseases, including skin diseases such as dermatitis and pemfigus; autoimmune disease such as systemic lupus erythematosus, polyarteritis and rheumatoid arthritis [10]. In hematology, CSs are combined with chemotherapy to treat lymphomas and leukemia as well as hemo-

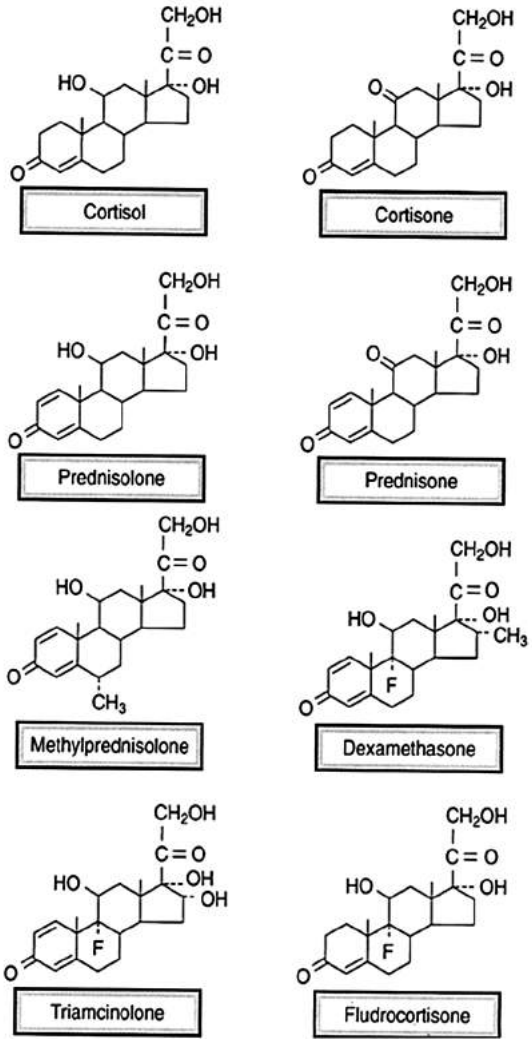


Figure 1. Chemical structure of the most commonly used glucocorticoids [6]
Slika 1. *Hemijska struktura najčešće korišćenih glukokordikoida [6]*

lytic anemia and idiopathic thrombocytopenic purpura [6, 11]. They are also applied in the treatment of gastrointestinal diseases such as inflammatory bowel disease, liver diseases (chronic active hepatitis), respiratory diseases (angioedema, anaphylaxis, asthma, sarcoidosis, chronic obstructive lung disease), etc. The mechanism of anti-inflammatory effect of CSs spreads on several levels and it has not yet been fully explained. On one level, CSs indirectly inhibit phospholipase A2 enzyme, which catalyzes the first degree of synthesis of inflammatory mediators, such as leukotrienes, prostaglandins and thromboxanes. On the second level, the activity of cycloozygenase (COX) of the key enzyme is inhibited in the synthesis of prostaglandin (inflammatory mediator). On the third level, CSs inhibit the activity of nitric oxide synthase (NOS)-, which is responsible for dilation of blood vessels in inflammation. Thus, CSs cause a reduced immunological response and alleviation or cessation of the inflammatory process.

Inhaled Glucocorticoids are used to treat the patients with asthma and chronic obstructive lung disease. Generally speaking, inhaled CSs have less frequent and milder side effects than oral and systemic CSs. However, there is a risk of developing adverse effects which depends both on the doses and the length of treatment. They are known to affect the bone metabolism; some studies have shown that the therapy with inhaled CSs is associated with a higher risk of fracture [12–14]. Besides the HPA axis suppression, which is caused by oral or systemic CSs, adrenal insufficiency has been shown to be associated also with inhaled CSs, but with a lower prevalence [15]. The risk is getting higher in the patients requiring long-term treatment with high doses of inhaled CSs, as well as in children [16, 17]. Chronic administration of inhaled CSs increases also the risk of developing cataract as well as pneumonia in patients having chronic obstructive lung disease [18, 19].

Intranasal Glucocorticoids are efficiently used to treat allergic rhinitis, rhinosinusitis, rhinoconjunctivitis and nasal polyposis [20, 21]. Intranasal CSs exert a localized activity with a minimal risk of systemic effect. No adverse effects have been detected in various studies [20]. However, it is recommended to avoid frequent and chronic use in order to prevent local and systemic complications [22].

Adrenal Insufficiency. Glucocorticoid therapy may cause numerous adverse effects such as a disorder in glucose metabolism, osteoporosis, higher risk of developing cardiovascular diseases, frequent infections, etc, the most common complication being adrenal suppression [23–26]. Iatrogenic or tertiary adrenal insufficiency is caused by chronic administration of high doses of corticosteroids [27]. From the physiological point of view, the hypothalamus secretes CRH which stimulates ACTH to be released from the anterior pituitary gland. ACTH leads to the release of cortisol from the zona fasciculate of the adrenal gland, which causes the negative feedback on the secretion of CRH and ACTH. Administration of exogenous CSs, even at low doses

Table 1. Glucocorticoids [4–6]
Tabela 1. *Glukokortikoidi [4–6]*

Glucocorticoids *Glukokortikoidi*	Equivalent doses (mg) *Ekvivalentne doze (mg)*	Anti-inflammatory (glucocortioid) potency *Antiinflamatorna (glukokortikoidna) potencija*	HPA suppression *HPA Supresija*	Mineralo-corticoid potency *Mineralo-kortikoidna potencija*	Plasma half-life (min) *Plazma poluživot (min)*	Biologic half-life (h) *Biološki poluživot (h)*
Short-acting/*Kratkodelujući*						
Cortisol/*Kortizol*	20.0	1.0	1.0	1.0	90	8-12
Cortisone/*Kortizon*	25.0	0.8		0.8	80-118	8-12
Intermediate-acting/*Srednje-dugo delujući*						
Prednisone/*Prednizon*	5.0	4.0	4.0	0.3	60	18-36
Prednisolone/*Prednizolon*	5.0	5.0		0.3	115-200	18-36
Triamcinolone/*Triamkinolon*	4.0	5.0	4.0	0	30	18-36
Methylprednisolone *Metilprednizolon*	4.0	5.0	4.0	0	180	18-36
Long-acting/*Dugodelujći*						
Dexamethasone/*Deksametazon*	0.75	30	17	0	200	36-54
Betamethasone/*Betametazon*	0.6	25-40		0	300	36-54
Mineralocorticoids/*Mineralokortikoidi*						
Fludrocortisone/*Fludrokortizon*	2.0	10	12.0	250	200	18-36
Desoxycorticosterone acetate *Dezoksikortikosteron acetat*		0		20	70	

HPA - hipotalamo-hipofizno-adrenalna osovina

Table 2. AI predictors and guidelines [3, 28–30, 33–36]
Tabela 2. *Prediktori adrenalne insuficijencije i preporuke [3, 28–30, 33–36]*

Possible HPA suppression *Verovatna supresija HPA*	Probable HPA suppression *Moguća supresija HPA*	Improbable HPA suppression *Nije verovatna supresija HPA*
Prednisone >20 mg/day time *Predinson ≥ 20mg/dan* Longer than 2 weeks/*Duže od 2 nedelje*	Prednisone <20 mg/day time/*Predinson <20mg/dan* Longer than 3 weeks/*Duže od 3 nedelje*	Any peroral dose/*Bilo koja peroralna doza* Less than 3 weeks/*Kraće od 3 nedelje*
Prednisone >5 mg/day time/*Predinson ≥ 5 mg/dan* Longer than 2 weeks/*Duže od 2 nedelje* Clinical signs of Cushing's syndrome *Klinički znaci Kušingovog sindroma*	Provided it has not been taken for more than 2 weeks in the evening/*Pod uslovom da nije uziman duže od dve nedelje uveče*	Prednisone <10 mg alternatively (not every day) *Prednison <10 mg naizmenično (ne svakog dana)*
Guidelines/*Preporučeni stav*		
No need for HPA axis evaluation *Nema potrebe za evaluacijom HPA osovine*	No need for routine HPA axis evaluation/*Nema potrebe za rutinskom evaluacijom osovine*	Treatment can be discontinued without special measures/*Tretman može biti prekinut bez specijalnih mera*
Should be treated as patients with secondary adrenal insufficiency/*Treba ih tretirati kao pacijente sa sekundarnom bubrežnom insuficijencijom*	Gradual discontinuation of treatment *Postepeno obustavljati tretman*	In severe cases but with caution/*Kod veoma teško obolelih sa oprezom*
Treatment should be discontinued gradually to enable HPA axis to recover/*Tretman je potrebno postepeno obustavljati da se omogući oporavak HPA osovine*	In case of abrupt discontinuation Synacthen test should be done *Ukoliko se naglo obustavi tretman uraditi Sinacthenski test*	
	If the patient is going to be exposed to acute stress (surgical intervention), Synacthen test should be done or stress doses of glucocorticoids should be administered/*Ukoliko će pacijet biti izložen akutnom stresu (hirurška intervencija...) uraditi Sinacthenski test ili tretirati sa stres dozama glukokortikoida*	

HPA - hipotalamo-hipofizno-adrenalna osovina

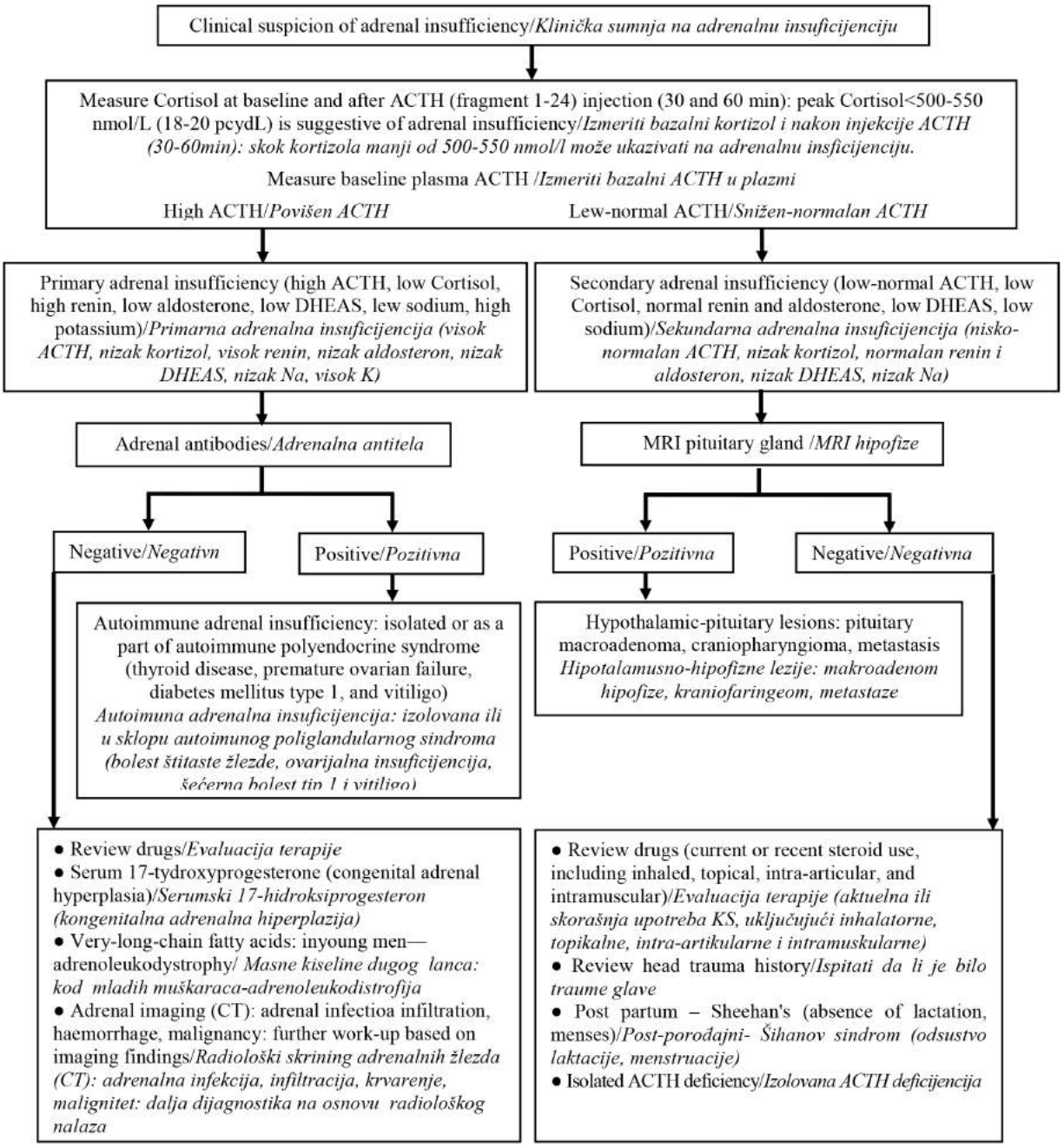
for only a few days, leads to the measurable suppression of the HPA axis by reducing the secretion of CRH as well as the synthesis and secretion of ACTH. Gradually, the anterior pituitary corticotropic cells will atrophy which will result in the absence of ACTH and the loss of adrenal cortex ability to produce cortisol [28]. The inhibition of the HPA axis function caused by CSs administration may last for 6 to 12 months once the treatment is discontinued [29].

There are several predictors of the potential glucocorticoid-induced adrenal suppression **(Table 1)**:

Table 3. Guidelines to reduce glucocorticoid dose [39]
Tabela 3. *Preporuke za smanjenje doza glukokortikoida [39]*

Initial dose of prednisone or equivalent dose *Inicijalna doza prednizona ili ekvivalent doze*	Recommended dose reduction *Predložena redukcija doze*
>40 mg/day/> *40 mg/dan*	5-10 mg/day every 1-2 weeks/*5-10 mg/dan svake 1-2 nedelje*
20-40 mg/day/*20-40 mg/dan*	5 mg/day every 1-2 weeks/*5 mg/dan svake 1-2 nedelje*
10-20 mg/day/*10-20 mg/dan*	22.5 mg/day every 2-3 weeks/*2.5 mg /dan svake 2-3 nedelje*
5-10 mg/day/*5-10 mg/dan*	1 mg/day every 2-4 weeks/*1 mg/dan svake 2-4 nedelje*
<5 mg/day/≤*5 mg/dan*	0.5 mg/day every 2-4 weeks alternatively 1 mg every second day *0,5 mg dnevno svake 2-4 nedelje/1 mg svaki drugi dan*

There are schemes to reduce the dose every second day or gradual reduction of hydrocortisone, which can be withdrawn when basal cortisol is >276 nmol/l; glucocorticoids can be necessary in stressful situations/*Postoje šeme sa smanjivanjem doze svaki drugi dan ili postepeno smanjivanje hidrokortizona koji može biti isključen kada je bazalni kortizol >276 nmol/l; glukokortikoidi mogu biti potrebni u stresnim situacijama.*



Scheme 1. The therapeutic algorithm of adrenal insufficiency diagnostics
Shema 1. *Terapijski algoritam dijagnostike adrenalne insuficijencije (ref. 34)*
ACTH - adrenokortikotropni hormon, CT - kompjuterizovana tomografija

– The kind of steroid use, i.e. their potency. As it has been shown in **Table 2,** long-acting CSs cause the suppression of the HPA axis much more often. Therefore, hydrocortisone and cortisone cause adverse effects least frequently; prednisone, prednisolone, methylprednisolone and triamcinolone are moderately suppressive whereas dexamethasone suppresses ACTH the longest.

– Systemic versus local therapy. Parenteral therapy has the highest potential to cause adrenal suppression [30–32].

– Daily therapy. There is evidence that the patients are at a lower risk of developing adrenal insufficiency if they take glucocorticoids every second day from the beginning of therapy [6, 28, 29].

– Split doses and night doses. CSs administration at several different doses during day causes higher risk of developing AI. Evening doses of glucocorticoids also tend to suppress normal ACTH secretion in the morning, which results in worse adrenal suppression. It is recommended to treat the patients with only one morning dose, i.e. a morning and afternoon dose whenever it is possible in order to imitate the natural circadian rhythm of cortisol secretion. Thus, it is recommendable to administer two thirds of the total daily dose in the morning and one third in the afternoon [30, 33, 34].

– The duration and cumulative dose of glucocorticoid treatment. Adrenal insufficiency is extremely rare in patients treated for a week or less [5, 36]. The so called short-term therapy, lasting up to 14 days, is also generally considered safe as is the case in the patients with acute exacerbation of chronic obstructive pulmonary disease [37].

– The best predictor of the HPA axis suppression is the immediate glucocorticoid dose [27]. There is a clear correlation between a dose of prednisone over 5 mg/day and impaired ACTH secretion [38]. Finally, it can be assumed that the patients at highest risk of developing AI are those who receive the highest doses (>20–30 mg of prednisolone or equivalent) of systemic CSs for a longer period of time (> 3 weeks).

Diagnosing Adrenal Insufficiency

Adrenal insufficiency is manifested by a number of symptoms and signs, the most common being fatigue, weight loss, appetite loss, nausea, vomiting, hyponatremia, hyperpigmentation and postural hypotension. **Scheme 1** shows the algorithm of AI diagnostics. Serum cortisol values above 496 nmol/l exclude the presence of AI, whereas those below 80 nmol/l (or below138 nmol/l according to some authors) confirm the presence of AI. Dynamic testing, such as insulin or Synacthen test, should be performed when the values of cortisol range between the above given ones. The presence of AI is also indicated by the cortisol values lower than 500 nmol/l. In addition to the low cotisol values in primary adrenal insufficiency, ACTH will usually exceed 100 pg/ml along with low aldosterone, high rennin and low dehydroepiandrosterone sulphate [1, 39].

Reduction in Glucocorticoid Therapy

Reduction or complete discontinuation of CSs is indicated once the maximum therapeutic benefit has been achieved or if considerable side effects have developed and become uncontrollable, such as diabetes mellitus, severe hypertension, osteoporosis, i.e. AI. The aim of the successful reduction in GC therapy is the period of gradual transition from the exogenous into endogenous corticolism of the organism without the reoccurrence of the underlying disease and AI or any other GC complication. Although there is no consensus in literature, several regimens have been published so far in clinical practice and the majority of doctors develop their own regimen of reducing therapy **(Table 3)**. Their common standing is that withdrawal from glucocorticostroid therapy should not be abrupt [29].

Conclusion

Biologically speaking, glucocorticoids are produced in the adrenal gland cortex and secreted into the systemic circulation. These steroids play a key role in the regulation of metabolism and functioning of cardiovascular system as well as in the response of the immune system to an inflammation. The major endogenous glucocorticoid in humans is cortisol, whose synthetic form is traditionally called hydrocortisone. Cortisone was first applied to treat rheumatoid in 1949. Since then, numerous synthetic compounds with glucocorticoid activity have been developed and they are used in treatment of a wide range of endocrine and non-endocrine diseases. Glucocorticoids can be administered systemically and locally (dermal, ophthalmological, inhalation, nasal or intra-articular application). A lot of side effects result from chronic administration of pharmacological doses of glucocorticoids. If a patient's health condition requires chronic use of glucocorticoids, intermediate-acting glucocorticoids are recommended to be used every second day to avoid potential complications. In addition, the patients must be monitored carefully and glucocorticoid therapy should be discontinued gradually to prevent adrenal insufficiency or reactivation of the disease under therapy.

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

4. Literatura

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

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

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

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

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

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

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

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

6. Dodatne obaveze

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

INFORMATION FOR AUTHORS

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

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

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

Manuscript submission should be made on the web address:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Preparation of the manuscript

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

The covering letter:

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

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

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

The manuscript:

General instructions.

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

The manuscript should contain the following elements:

1. The title page.

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

2. Summary.

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

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

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

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

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

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

3. The text of the paper.

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

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

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

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

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

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

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

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *A computer file*

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

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

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

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

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

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

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

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

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

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

6. Additional requirements

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